THE OCCURRENCE AND MOVEMENT OF *FRANCISELLA TULARENSIS* MCCOY AND CHAPIN ACROSS LANDSCAPES

A Dissertation

by

KEITH WAYNE BLOUNT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Entomology

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Approved by:

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ABSTRACT

The Occurrence and Movement of *Francisella tularensis* McCoy and Chapin Across Landscapes. (December 2007)

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Tularemia is a one of the most complex zoonotic diseases. *Francisella tularensis* McCoy and Chapin, the causative agent of tularemia is considered endemic in Texas, but outbreaks are rare and there are few human cases each year. Tularemia is listed as a Category A biological weapon and air samples are taken daily in select major metropolitan areas, including Houston, to monitor for its presence. I determined the potential risk for tularemia introduction and spread in southeast Texas through field surveillance for the pathogen and its major arthropod vector in the region, *Amblyomma americanum* (L.); completion of a habitat capability map for *A. americanum*, based on landscape analysis of the study area; and potential movement and long-term establishment of tularemia through development of a spatially explicit, agent-based, simulation model.

Field and laboratory investigations resulted in the identification of two samples positive for *F. tularensis*. A feral cat tested positive for Type B tularemia using a new aptamer-based assay, and one sample returned positive in *Amblyomma maculatum* by polymerase chain reaction. This work sheds light on a complex host-pathogen-vector

interaction in the rural to urban interface and establishes a framework for future tularemia field work and pathogen modeling in the rural to urban interface.

DEDICATION

This work is dedicated to Dr. Cluff Hopla, Professor Emeritus at the University of Oklahoma. Though I haven't had the honor of meeting Dr. Hopla, I feel like I have known him in some respects. Both Dr. Pete Teel and Dr. Ken Gage (a student of Dr. Hopla) worked with Dr. Hopla for many years, and in turn, have surely passed on lessons learned. Dr. Hopla's work on the ecology of tularemia may be thirty years old and the transmission studies of *Francisella tularensis* in ticks and mammals are over fifty years old, but they are still as relevant today as they were decades ago. I seem to pick up something new every time I read them. For the years of hard work and well written papers – thank you, we will try to carry the torch in your honor.

ACKNOWLEDGMENTS

I thank my committee, Dr. Pete Teel, Dr. Jimmy Olson, Dr. Mike Longnecker, Dr. Johnathan Kiel, Dr. Ken Gage, and Dr. Bill Grant, for their support and encouragement.

In particular, I thank Dr. Pete Teel for having the faith to support what some might have viewed as a potentially ambiguous, poorly defined, unattainable, research project. You are a great teacher, researcher, and administrator (yes, this is notable). You are an even better man than I had heard you were, and I am proud to be able to call you my mentor and friend.

Dr. Bill Grant is a systems modeler extraordinaire and one of the nicest guys one could ever work with. Your insight into systems modelling is unparalleled. Your help and guidance throughout this project was greatly appreciated. I even forgive you for making me listen to all your jokes – three times (good luck with that Todd). You made complex topics (systems modeling, mammal movements, how much coffee one should drink in a day, which brands of chocolate are best) easy enough that even a military guy could get it.

I thank the Air Force Research Laboratory for field and laboratory support. In particular, Captains Wes Walker and David Sanders, who worked many long hours in the south Texas sun and occasionally, endured venomous creatures being tossed their way.

This project had involvement and assistance at all levels from multiple organizations and agencies. The following people and organizations provide time, laboratory, equipment, and personnel support:

- The CDC-Fort Collins (Dr. Ken Gage) and CDC-Atlanta (Dr. Amanda Loftis) for their support and training, not only my training but my Air Force counterparts in San Antonio.
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- Harris County Mosquito Control (Christina Hailey-Dischinger for GIS support and equipment)
- Texas Department of State Health Services (Dr. Paul Grunenwald, Jael Miller)
- USDA-APHIS Wildlife Services (Janean Romines, Dee Manshack)
- Knowledge Engineering Laboratory, Texas A&M University (Maria Tchakerian)

I thank the Air Force for giving me this educational opportunity. I have the best job and serve the greatest country in the world.

To my wife, Pam, you support me every day, keep me focused, and love me unconditionally in spite of all my faults. To my girls, Sara and Kristen, you make me laugh and give me a reason to work hard and play hard. To Mom, you always supported

and encouraged me with idiomatic expressions like “it’s no step for a stepper”. Thank you.

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CHAPTER I

INTRODUCTION

Tularemia is a zoonotic disease caused by the bacterium, *Francisella tularensis* McCoy and Chapin. This pathogen is extremely virulent and infections may be air-borne, food/water-borne, vector-borne, or result from direct contact with tissues of infected animals (Feldman et al. 2001). Recently, the complete genome sequence of a highly virulent form of *F. tularensis* was completed (Larsson et al. 2005), as well as a detailed molecular analysis of *F. tularensis* in the U.S. (Staples et al. 2006). Current research supports historical evidence that two subspecies of *Francisella tularensis* cause disease in the United States: *tularensis* (Type A) and *holarctica* (Type B), both differing in geographic distribution and virulence (Ellis 2002).

Over 150 species of vertebrate animals can be infected with *F. tularensis*. Both domesticated and wild animals are affected, some may serve as sources of infection but little is known about the true reservoir potential. The ease at which *F. tularensis* can move among animal populations became evident when naturally infected prairie dogs, *Cynomys ludovicianus*, were shipped from South Dakota to Texas, West Virginia, and the Czech Republic, inadvertently moving diseased animals across state boundaries and international borders (Petersen et al. 2004b). Rodents and lagomorphs appear to be important vertebrate reservoirs for this disease agent in the United States. A clearer

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This dissertation follows the format and style of The Journal of Medical Entomology.

genetic and epidemiological picture of *F. tularensis* is emerging, but the ecology of *F. tularensis* is poorly understood and has not been thoroughly investigated in over thirty years (Hopla 1974, Jellison 1974).

Human cases of tularemia in the southeastern U.S. most often have been the result of contact with infected lagomorphs (rabbit hunting) or direct infection via tick bite (Taylor et al. 1991, McChesney and Narain 1983). There is a bimodal distribution of cases that clearly reflects the seasonality and two primary routes of exposure: hunting/trapping in the fall/winter and exposure to ticks in the spring/summer (Assal et al. 1967). As a result, tularemia has been defined in terms of its epidemiology rather than its ecology. The importance of this recognition is evident as one attempts field studies, surveillance, or explanations of outbreaks of *F. tularensis*. Even though human cases result from contact with lagomorphs, the status of lagomorphs as the primary reservoir of infection is unproven.

There is substantial evidence that lagomorphs may not be the maintenance reservoir based on susceptibility to infection and the epizootic potential of *F. tularensis* on lagomorph populations once introduced (McCahan et al. 1962). In lagomorphs, *F. tularensis tularensis* (Type A) has an LD_{50} of 10 organisms (Dennis 1998). Contrary to early held beliefs that lagomorphs mounted little to no immune response to *F. tularensis*, it is evident from more recent work that lagomorphs do indeed possess a humoral response and antibody titers rise and fall in response to exposure (Shoemaker et al. 1997, Lepitzki et al. 1990). Even though some lagomorphs appear to be able to clear infection, most *F. tularensis* introductions are devastating to the population (Woolf et al. 1993).

Scientists in the United States, Japan, and the former Soviet Union studied *Francisella tularensis* for offensive purposes as a biological weapon. The physical and biological properties of *F. tularensis* were changed in order to make it more lethal and weapon capable, a process often referred to as "weaponization". Formulations were created which allowed the weaponized pathogen to be disseminated by aerosol delivery systems. There is also evidence to suggest strains were modified to be vaccine and antibiotic resistant (Dennis et al. 2001).

Pathogenic organisms and toxins that may be used as biological weapons against humans or agriculture are governed by the U.S. Departments of Health and Human Services (DHHS) and Agriculture (USDA), respectively. *Francisella tularensis* is currently listed as a Category A select agent, which means its possession, use, and transfer is regulated by the Centers for Disease Control and Prevention (CDC 2007). *F. tularensis* warrants its position as a Category A select agent because of its high infectivity and ease of dissemination. Extensive research and preparedness continues on tularemia as it impacts human health. A World Health Organization report estimated that an aerosol release of *F. tularensis* in a city of 5 million would cause 250,000 casualties and 19,000 deaths (WHO 1969). However, little research has been published as to the impact of tularemia on non-human animals, or the fate of *F. tularensis* in the environment (Dennis et al. 2001). Since the primary route of entry for weaponized tularemia is respiratory, if tularemia were released, many non-human animals would be exposed and potentially become infected.

The toll to companion animals, agricultural production animals, and wildlife populations from the release of a zoonotic pathogen is unknown. Some animals may be inherently resistant or refractory to disease due to previous exposure from natural zoonotic cycles, and thereby function as competent reservoirs (Petersen et al. 2004a). The epidemiological function of vectors or other ectoparasites is also unknown. Arthropods may become infective due to feeding on infected and moribund animals, potentially increasing the spread of a pathogen by normal propagative transmission modes, or they may spread *F. tularensis* by mechanical means.

Ticks and biting flies are competent vectors of tularemia and historically have accounted for the majority of cases of tularemia in the United States (Jellison 1974, Klock et al. 1973). However, most clinical cases are tied back to arthropod exposure through epidemiological questioning (history of tick or fly feeding) and not isolation of the pathogen from the vector. The evidence for vertical transmission of *F. tularensis* in field and laboratory settings is unconvincing. Some ticks maintain *F. tularensis* infection by transstadial and transovarial transmission; however, their importance in the maintenance and reservoir status is unclear (Parker et al. 1924, Burgdorfer and Varma 1967, Hopla 1974). The exact mode of transmission within ticks is also unresolved and likely differs for each tick species, depending on feeding patterns and host factors (Hopla and Hopla 1994). The potential for arthropods to take up modified pathogens and move them to new locations or to serve as pathogen reservoirs and begin new cycles of disease after an initial outbreak is speculative. The fate of modified pathogens in the

environment and potential for disease in non-target animals is unknown and an area greatly in need of study.

The context of questions surrounding the persistence and dispersal of a biological agent, released in an urban setting and potentially spreading to rural settings, challenges all that is known about the ecology and epidemiology of these pathogens. There is a growing need for understanding how pathogens, historically causing rural diseases, interact in an urban setting. According to the Census Bureau, urban areas are all territories, population, and housing units in densely settled areas with a population density of at least 1,000 people per square mile. Rural areas are defined by the U.S. Census Bureau as areas that lie outside of the urban areas or urban clusters. Urban areas are characterized by intense development and high population density. In addition to lower population density, rural landscapes are generally characterized by more vegetation and agricultural ecosystems, and less infrastructure and development. Plant and animal communities differ between urban and rural areas. Animal ranges and dispersal are different in rural versus urban settings (Dykstra et al. 1997, Gaughan and Destefano 2005). Defining and studying these heterogeneous land areas, the interacting ecosystems, and the flow of energy, materials, and species are the bases for landscape ecology (Forman 1995).

Using the landscape perspective to describe disease trends and associations in terms of patches, corridors, and surrounding matrix is the basis for landscape epidemiology (Kitron 1998), a field that is greatly enhanced by GIS (geographic information systems) functionality (Daniel et al. 2004). These tools are frequently used

to establish links between epidemiological and spatial data. Since arthropod distributions are inherently spatial and tick mobility is limited to their hosts, tick-borne diseases were among the first arthropod vectored diseases to be analyzed using GIS and remote sensing (Randolph 2000). The associations between ticks and their habitat are well known (Needham and Teel 1991). Risk assessments of Lyme Borreliosis and other tick-borne diseases using these GIS tools have been completed (Guerra et al. 2002, Randolph 2000, Eisen et al. 2006).

One of the results of human activity across any landscape is increasing fractionation by creating networks (roads, power lines, fences, land boundaries, aqua ducts) across the given landscapes resulting in smaller contiguous homogeneous areas (patches). Burgeoning human populations and the associated growth spreading to outlying rural areas is known as urban sprawl. Urban sprawl is a phenomenon that has public health consequences. Rural populations generally are thought to be in poorer health and have less access to health care than their urban counterparts (Blumenthal and Kagen 2002). Lifestyle differences of a rural population also may expose this group to a wider array of zoonotic pathogens. Risk factors, such as animal production and hunting, expose rural populations to pathogens not typically encountered by their urban counterparts. Epidemics of tularemia have been shown to be directly related to hunting and farming (Stewart 1996). Immunologically naive suburban populations, situated between the two extremes of exposure, should be more at risk of coming into contact with animals and their pathogens than their urban counterparts. This results in the three subpopulations falling into differing categories: urban - susceptible but not exposed;

rural - exposed and not susceptible; suburban - susceptible and exposed. Morse (1995) identified factors, including changing agricultural practices and urbanization, as major contributors to the emergence of infectious disease.

In addition to risk from naturally occurring diseases, there is an increased risk of exposure to intentionally released pathogens. Many of the agents of greatest concern cause diseases that have appeared throughout history and still cause epizootics throughout the world. Pathogens used as biological weapons also could differ from their natural counterparts in several ways, most importantly in their pathogenicity. For example, the genetic makeup of these pathogens may be modified, resulting in vaccine/antibiotic resistance or toxin production. These chimerical creations may or may not interact with the environment and hosts in predictable ways. What is more predictable is how these biological weapons will be used, or more precisely, on what population they will be used. It is clear that terrorism is more effective against large urban populations. One of the primary goals of terrorists is to disrupt society and cause fear and panic in a society. Clearly, a terrorist act in Washington D.C. has more impact than one in rural Oregon. Therefore, there is a real need to understand how natural or modified zoonotic pathogens released in an urban/suburban setting might move throughout the landscape.

Models can be a valuable component to ecological studies. They are essential to bioterrorism preparedness. Most pathogens and toxins that have been modified, or are good candidates for use as a weapon, are categorized by the Centers for Disease Control and Prevention on the Select Agent list. The CDC Select Agent Program oversees

activities and registers all laboratories and other entities in the United States that possess, use, or transfer a select agent or toxin (CDC 2007). The implication to the research and medical community is that by working with a select agent, one can no longer maintain or transport viable samples without strict federal government oversight. These regulations are necessary given the recent history with anthrax and the current world situation, but the additional oversight likely discourages new research and field studies in the ecology and epidemiology of the zoonotic pathogens. As a result, bioterrorism agents are prime candidates for simulation modeling and there are a number of GIS-based simulation models available to government officials and the research community for such modeling.

One of the models used by the Department of Homeland Security and Department of Defense is called HPAC (Hazard Prediction and Assessment Capability). HPAC was developed by the Defense Threat Reduction Agency for use in calculating transport and dispersion of materials (chemical, biological, and radiological) in the atmosphere (Sykes and Gabruk 1997). The output of the HPAC model for a biological agent is a map showing contours of the area with probability of injury, fatality/mortality, and infection. HPAC was used at the beginning of this project to provide a general idea of what size area might be affected if a biological weapon, like *F. tularensis*, were released in urban Houston, TX. Results of model runs using default parameters and real-time weather data for the area showed the area of coverage could easily reach from the Houston ship channel to the piney woods region of east Texas, covering hundreds of square miles and affecting thousands of people (Fig. 1.1). This provided the frame-work

for the potential problem and established that, in theory, a biological weapon release could easily span the urban-suburban-rural interface.

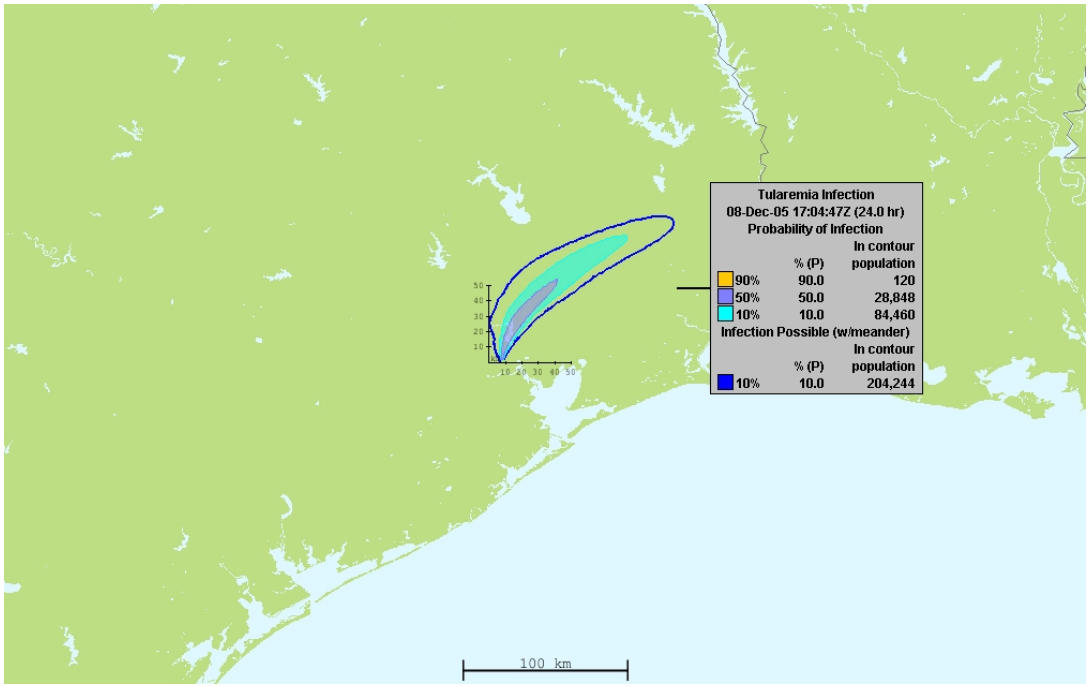


Figure 1.1 Hazard Prediction and Assessment Capability (HPAC) model results of *F. tularensis* release in Houston, TX**.**

Response and preparedness to bioterrorism and the introduction of zoonotic pathogens are a major component of public health infrastructure. The anthrax attacks in the United States in 2001 solidified the reality of bioterrorism for this country and generated a new awareness for the need for appropriate public health response. Local, state, and federal officials now share responsibility in what was once considered primarily a military issue. The key preventive medicine component to the response

capability is disease surveillance. Environmental surveillance includes both biotic and abiotic factors. Water, soil, air, arthropod, and animal samples are most important. Information from these samples is used to promulgate disease control and prevention plans. There is currently no official, widely available, surveillance plan that addresses the long term environmental impact to the introduction of weaponized zoonotic pathogens.

To meet the challenges presented by the potential introduction of *F. tularensis*, this project addressed specific short term and chronic surveillance problems. The goal of this project was to add to the current body of knowledge regarding the ecology of *F. tularensis* and provide a framework for further field studies to approach this challenging concept. Using a landscape epidemiological approach, I created a spatially-explicit simulation model that could serve as a tool to aid in surveillance and control of arthropod vectors and animal hosts/reservoirs in the event of a natural epidemic or outbreak due to a bioterrorist attack employing *F. tularensis* or similar agent.

CHAPTER II

SURVEY OF HOSTS/VECTORS FOR *FRANCISELLA TULARENSIS* IN SOUTHEAST TEXAS

Type A tularemia, *Francisella tularensis tularensis*, is generally regarded as a rural disease of lagomorphs and rodents transmitted by ticks (Ixodidae) and/or flies (Tabanidae). Type A is found in North America only. Type B tularemia, *Francisella tularensis holarctica*, is found in the Northern Hemisphere, is less virulent than type A, and in the U.S. is primarily associated with aquatic rodents and occasionally arthropod vectors (Feldman 2003, Farlow et al. 2005). A simplified epidemiological picture of the host/pathogen/vector interaction is often portrayed with a single arthropod vector and a single mammalian source of infection. If one considers all the potential vectors, hosts, pathogens, and their respective interactions in the varied environments where this disease is found, one could argue that tularemia is the most complex zoonotic disease known.

The concept of tularemia as a disease of lagomorphs and ticks fails to encompass the true complexity of the ecology and epidemiology of the pathogen and the disease. A more realistic, yet still incomplete, ecological picture for *F. tularensis* (Hopla and Hopla 1994) includes over 250 vertebrates and invertebrates, covering a broad array of taxonomic groups, both aquatic and terrestrial (Burroughs et al. 1945, Hopla 1974, Sjostedt 2005). How the pathogen maintains itself in nature, and particularly the

complex interactions required of arthropods and vertebrates, is complicated and poorly understood (Petersen et al. 2004a, Farlow et al. 2005, Eisen 2007).

The ecology of tularemia in the U.S. has not been updated since the early 1970s (Hopla 1974, Jellison 1974). Current research efforts focus more on tularemia epidemiology, molecular characterization, and clinical treatment options (Farlow et al. 2005, Staples et al. 2006). The U.S. averages fewer than 200 cases of tularemia per year. Although Texas is not within the hyperendemic area, tularemia cases in humans, wild and domestic animals are not uncommon (Avashia et al. 2004). There are no clear trends for tularemia in Texas within the last 20 years, primarily because reporting of the disease has been inconsistent. Human cases were reportable to the CDC up to 1993, but tularemia was removed from the list of reportable diseases in 1994 and remained there until 2000, when it was reinstated due to concern over its use as a bioterrorism weapon (Dennis et al. 2001). Texas started tracking tularemia cases again in 2002 and for the past nine years where data were available (1990-1993, 2002-2006) there were 17 cases of tularemia reported in Texas. The Houston area (Harris County) produced more tularemia cases than any other location in Texas during the years 1990-2000 (MMWR 2002).

The Houston/Harris County area is unique not only because it has a history of producing tularemia cases, but because of its geographic position. It is a large port city with heavy commercial traffic along rail, air, road, and sea routes – a point of significance that led the Department of Homeland Security to select sites for BioWatch sensors within this city/county. The Houston/Harris County area is connected to the

tularemia endemic Ozark Mountains by heavily forested areas along eastern Oklahoma, western Arkansas, and east Texas. This woodland corridor provides a nearly uninterrupted route from the highly endemic areas for tularemia all the way to Sam Houston National Forest and the suburban outreaches of Houston and southeast Texas. By the same token, if tularemia or a similar zoonotic pathogen were introduced into the Houston area, it is possible that the movement of that pathogen via hosts and vectors could infiltrate from areas of low endemicity (southeast TX) to areas of high endemicity (Ozark region).

Tularemia was first described as a "plague-like disease of rodents" (McCoy 1911). Since its original naming of *Bacterium tularense* (McCoy and Chapin 1912), there has always been an association between rodents and *F. tularensis* but the importance of rodents in the overall maintenance of the disease has typically been underappreciated when compared to that of lagomorphs. Many clinical cases are tied to exposure to lagomorphs, so it is easy to understand how their maintenance role could be elevated. Hopla (1974) summed up our anthropocentric view in his seminal ecologic work when he stated, "Much that has been written about tularemia concerns its effect upon man; yet, man is incidental to the course of tularemia in nature." The focus of attention during epizootics of tularemia on humans may mask the underlying nature of the maintenance of this disease.

The contribution of non-lagomorph vertebrates as sources of *F. tularensis* during inter-epizootic periods may be significant. Bell and Stewart (1975) were able to show that *Microtus pennsylvanicus* sheds *F. tularensis* in the urine, and could serve as a

source of infection for Type B tularemia. More recently, *F. tularensis* was isolated from the deer mouse, *Peromyscus maniculatus*, during a population explosion and subsequent die-off (Wobeser et al. 2007). Quite understandably, declining populations in lagomorphs would be more visible, especially to home owners or hunters, than that of voles and mice. The latter are small, nocturnal and secretive, all of which might lend to their being overlooked during epizootics and subsequent die-offs. It is possible their role in tularemia ecology is underappreciated since they are not directly involved with human cases in the U.S. or visible as possible sources of infection for *F. tularensis*.

Our understanding of the role arthropod vectors play in *F. tularensis* ecology exceeds that of other components, thanks to the early work of pioneers like R. R. Parker and C. E. Hopla, whose pathogen transmission work paved the way for a better understanding of the ecology of tularemia (Parker et al. 1924, Hopla 1955). However, there are far more questions than answers regarding tick-borne transmission of *F. tularensis* and there are calls from other investigators for renewed research on this disease (Eisen 2007).

In the southeastern United States, no other tick is more commonly encountered by humans than the lone star tick, *Amblyomma americanum* (L.) (Campbell and Bowles 1994, Merten and Durden 2000, Childs and Paddock 2003). This species has a broad host range and can be found in great numbers when encountered with its primary host, the white-tailed deer, *Odocoileus virginianus* (Zimmermann) (Patrick and Hair 1978). For example, Brennan (1945) reported that in the summer of 1943, four individuals collected over 4,000 lone star ticks under one tree near San Antonio, Texas. *A.*

americanum is a known vector for *F. tularensis* (Hopla 1953,1955). The lone star tick is also known to vector or harbor *Coxiella burnetii, Ehrlichia* spp., *Rickettsia* spp., and *Borrelia lonestari* (Brennan 1945, Sonenshine 1993). The status of *A. americanum* as a vector of pathogens of public health significance, and not merely a pest of wild and domestic animals, and humans, is no longer in question (Childs and Paddock 2003). This chapter reports the findings of a survey to determine the occurrence of *F. tularensis* and other pathogens in ixodid ticks and host animals in urban, suburban, and associated rural areas of Houston, TX.

Materials and Methods

Study Area

The study area consisted of sites in southeast Texas, in or near the Houston area. Sites were selected based on appearance of habitat (likelihood of producing ixodid ticks), history of tick complaints (personal communication with epidemiologists at Texas Department of State Health Services), and access. Initial samples were collected from Harris, Montgomery, Walker, and Liberty counties. Follow-up and/or multiple samples were collected from three main areas: Harris County (urban site), Montgomery County (suburban site), and Walker County (rural site). Sites were located within three vegetation regions: Pineywoods, Gulf Prairies and Marshes, and Post Oak Savannah. Complete descriptions of the vegetation communities are found in Gould (1975) and Jones et al. (1997). Sites were defined as rural, suburban, or urban based on field evaluation, proximity to Houston, and landscape metrics.

Tick Collections

Ticks (Ixodidae) were collected using a variety of methods, depending on location and species. Since the lone star tick was the primary arthropod of interest, the carbon dioxide (CO^2) baited trap was employed for most collections. Trap description and trapping methodology was employed as previously described (Fleetwood et al. 1984). Collections using tick drags were made in some locations due to variation in attractiveness to carbon dioxide by different tick species (Ginsberg and Ewing 1989, Kinzer et al. 1990). This was either done in conjunction with other collection methods or as a stand-alone method of tick surveillance in an area. For example, in some areas where red imported fire ants, *Solenopsis invicta* Buren, were very common, a tick drag was employed rather than a CO2 trap to avoid fire ant stings, or lengthy removal of ants from the tick traps. In some locations, tick drags were used to scout potential sites for further trapping. All ticks, regardless of collection method, were placed in reagent grade (absolute) ethanol until further processing.

Host Animals

Ectoparasites and/or tissue samples were collected from a variety of mammals at different sites. The majority of the specimens collected and tested were obtained through cooperative agreements with USDA-APHIS Wildlife Services and the Harris County Public Health and Environmental Services (formerly Harris County Animal Control, HCAC). Animals were collected during routine duties (i.e., animal control

officers catching feral animals in Harris County and Wildlife Services personnel trapping nuisance animals) and processed at selected sites. Members of the U.S. Air Force Research Laboratory (AFRL), San Antonio, TX, collaborated in trapping and processing ticks and rodents, as well as processing at AFRL facilities. Attempts were made, unsuccessfully, to trap rodents using standard trapping methods (Sherman traps, H.B. Sherman Traps, Tallahassee, FL) using protocols established by the Centers for Disease Control and Prevention (CDC) (Mills et al. 1995).

Testing Methods

Testing of diagnostic specimens was supported by AFRL and the CDC. When possible, more than one method for detection of *F. tularensis* was used. The accepted diagnostic test for detection of *F. tularensis* is culture and/or polymerase chain reaction (PCR) (Petersen et al. 2004b). Culture recovery of *F. tularensis* is inherently problematic due to the fastidious growth of the organism and the danger of infection to laboratory personnel (Burke 1977, Shapiro and Schwartz 2002). Only registered laboratories may culture *F. tularensis* due to the Select Agent rules (DHHS 2005), therefore, no attempt was made to confirm field results by culture.

The primary method used for detection of *F. tularensis* during this research project was PCR. Some research shows PCR to be superior to culture for detection of *F. tularensis*, as it is more sensitive and indicates infections in individuals that do not seroconvert (Johansson et al. 2000). That stated; there are limitations to using PCR for confirmation of an organism. Therefore, it was important to have serological results to

complement molecular results when possible. PCR assays were conducted by CDC-Atlanta, GA, and AFRL personnel, respectively. A novel technique, ALISA (aptamer-linked immobilized sorbent assay), was conducted by AFRL. Serology (microagglutination) was accomplished at CDC-Fort Collins, CO.

PCR and RFLP

DNA from ticks was extracted as described by Loftis (2005). Ticks were removed from alcohol, frozen in liquid nitrogen, and crushed using sterile Teflon pestles. The Iso-Quick nucleic acid extraction kit (Orca Research, Inc., Bothell, WA) was used for DNA extraction. *F. tularensis* was detected using a nested PCR for the *FopA* gene (Fulop et al. 1996). *Rickettsia* was detected using a nested PCR for the 17-kDa gene and a direct PCR assay for the rOmpA gene (primers17kDF1/17kDR1) (Carl et al. 1990, Roux et al. 1996). Ticks were screened for *Ehrlichia* as described by Loftis (2006). Ticks were screened for *Borrelia* using a nested PCR assay for the flagellin gene (primers FlaLL/FlaRL and FlaLS/FlaRS) (Barbour et al. 1996). All PCR assays were performed using the following protocol:

Taq Master Mix (Qiagen)

1 uM each primer (synthesized at CDC)

Single round assay: 2uL DNA in 20 µL final reaction volume

Nested assay: 1uL DNA in 10 µL primary reaction, then 1.5 µL primary product in 20 µL nested reaction volume

Thermocycler program was: 95°C for 3 min, followed by 40 rounds of 95°C for 30 s, *°C for 30 s, and 72°C for 60 s, and a final extension of 5 min at 72°C. *Annealing temp based on the published optimum temperature for the assay. Products were separated using 10 µL of the amplicon in loading dye (30% glycerol in water + xylene cyanol + bromophenol blue), on 1% agarose gels (LE agarose, Promega) in TAE buffer at 140V for 30 min and visualized using ethidium bromide and UV light.

Amplicons produced by the 17-kDa antigenic gene PCR for *Rickettsia* were further characterized using restriction fragment length polymorphism (RFLP) analysis under the following conditions:

5 µL of PCR product in 10 µL final volume containing 1 unit of each restriction enzyme (New England Biolabs) in the manufacturer's recommended/provided buffer for 4-6 hrs at 37°C.

Products (all 10 µL) were mixed with loading dye and separated on 4% agarose gels in TAE with ethidium bromide.

Sequencing of PCR products was performed as described by Loftis (2005).

Microagglutination (MA) Test for Antibodies to *Francisella tularensis*

Sera from selected samples were examined for the presence of antibodies against *F. tularensis* at the CDC-Fort Collins, CO. Sera were collected in the field on specialized filter paper (Nobuto strip type 1, Toyo Roshi Kaisha, Ltd., 1-5-10, Kotobuki, Taito-ku, Tokyo 111-00042 Japan), air dried, and stored in individual paper envelopes until testing. The MA test was completed under the supervision of trained laboratory

personnel at the Bacterial Zoonoses Branch of the CDC, and in accordance with previously described procedures (Brown et al. 1980, Chu 2000).

ALISA

Single-stranded oligonucleotides molecules (aptamers) were used to screen for *F. tularensis*. This novel detection approach was recently described (Vivekananda and Kiel 2003). Sera from mammals (collection described above) were grown in brain heart infusion media with 0.1% cystine at 37°C with 5% CO^2. Samples were boiled for 1 hour after 48 hours growth, and then centrifuged at 6000 rpm for 30 min. The bacterial pellet was washed with phosphate-buffered saline (PBS) once, then resuspended in PBS, and stored at -80°C until the ALISA was performed. This assay has high specificity and affinity for *F. tularensis,* with detection limits as low as 25 ng of bacterial antigen (Vivekananda and Kiel 2006).

Results

Tick Collections

In June and August 2005, there were 1,860 ticks collected from 130 CO_2 traps (14.31 ticks/trap) from three sites. The trapping locations were all located in densely wooded areas within the Sam Houston National Forest or Huntsville State Park, TX. The ticks collected from these rural sites were almost exclusively (1858 of 1860) *A. americanum*. Larval ticks were not identified to species. However, given the location and time of year, it is believed these ticks were *A. americanum* larvae. Two adult Gulf Coast ticks, *A. maculatum*, were also collected (Table 2.1).

In 2006, 82 ticks were collected from CO_2 traps and tick drags. The focus of tick collections in June was urban and suburban sites in and around Houston, TX. Of the twenty ticks collected in June, eighteen (90%) were adult *A. maculatum*. One was *A. americanum* and the other was *D. variabilis* retrieved from a tick drag. The twenty ticks were collected from 197 CO_2 traps, for a ticks/trap rate of 0.10. In October, 62 ticks were collected from 341 CO_2 traps at Camp Strake, a Boy Scout camp at Conroe, TX. The ticks/trap rate at Camp Strake, a suburban site, was 0.18. Representative species for October collections were *A. americanum*, *A. maculatum*, *D. variabilis*, and *I. scapularis*. The breakdown of tick species and numbers collected in 2006 is found in Table 2.2.

Table 2.1. Species and abundance of ticks collected in June and August 2005 from Sam Houston National Forest and Huntsville State Park, TX.

Tick Identification	Larvae	Nymph	Adult Female	Adult Male
Amblyomma spp.	804	-	-	-
A. americanum	0*	1041	5	8
A. maculatum	0*	0	1	1

* Larvae were identified only to Genus level.

Table 2.2. Species and abundance of ticks collected in June (Houston, TX) and October (Conroe, TX) 2006 from carbon dioxide baited traps and tick drags.

Tick Identification	Larvae	Nymph	Adult Female	Adult Male
Amblyomma spp.	4	0	0	0
A. americanum	0*	39	4	0
A. maculatum	0*	0	3	17
Dermacentor variabilis	0	0	2	0
Ixodes scapularis	0	0	4	9

* Larvae were identified only to Genus level.

Identification of Agents from Ticks

Ticks were collected, identified, and sent to the CDC-Atlanta for testing. Of the 167 individual ticks or pools of ticks tested, almost half were found to harbor either potentially pathogenic organisms or endosymbionts. *Rickettsia amblyommii* was the most common organism identified from the samples. *R. amblyommii* was found in 28 of 72 (38.9%) *A. americanum* adults and 39 of 52 (75%) *Amblyomma* nymphs. It was also found in one *A. maculatum* adult. Three *A. americanum* adults and one nymph were coinfected with *Borrelia lonestari* and *R. amblyommii*. Two adult *A. americanum* were infected with *R. canadensis*. One *Ixodes scapularis* was infected with *Ix. scapularis* symbiont, one was infected with *R. cooleyi*, and two were coinfected with those two organisms. One adult *A. maculatum* was infected with an unnamed *Rickettsia* from the Pete Teel colony at Texas A&M University. Last, but certainly not least, one adult male *A. maculatum* collected in June 2006 was infected with *Francisella tularensis* and Panola Mountain *Ehrlichia*. The results of screening ticks for pathogens/endosymbionts are listed in Table 2.3. The GenBank accession numbers for pathogens identified are listed in Table 2.4.

Table 2.3. Pathogens identified from ixodid ticks collected in southeast Texas in 2005-2006.

Tick species ID	Rickettsia species ID	Tularemia ~400bp	Borrelia Fla 350bp,600bp	P.Mtn. Ehr
A. americanum		-	-	-
A. spp.		-	+	ND
A. americanum	R. canadensis-like	-	-	-
A. americanum	R. canadensis-like	-	-	-
A. maculatum		-	-	-
A. spp.	"R. amblyommii"	-	+	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. americanum		-	-	-
A. americanum		-	-	-
A. maculatum	Pete Teel colony Rickettsia	-	-	-
A. americanum		-	-	-
A. spp.		-	-	ND
A. americanum	"R. amblyommii"	-	-	-
A. spp.	"R. amblyommii"	-	smear	ND
A. spp.	"R. amblyommii"	-	-	ND
A. americanum		-	-	-
A. spp.	"R. amblyommii"	-	-	ND
A. americanum	"R. amblyommii"	-	+	-
A. americanum	"R. amblyommii"	-	+	-
A. americanum	"R. amblyommii"	-	+	-
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. americanum		-	-	-
A. spp.	"R. amblyommii"	-	-	ND
A. americanum	"R. amblyommii"	-	-	-
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. americanum		-	smear	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. spp.		-	-	ND

Table 2.3 Continued.

Tick species ID	Rickettsia species ID	Tularemia ~400bp	Borrelia Fla 350bp,600bp	P.Mtn. Ehr
A. americanum	"R. amblyommii"	-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. spp.	"R. amblyommii"	-	-	ND
A. spp.		-	smear	ND
A. spp.	"R. amblyommii"	-	-	ND
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
R. sanguineus		-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
D. variabilis		-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. americanum	"R. amblyommii"	-	-	-
A. spp.	"R. amblyommii"	-	-	ND
D. variabilis		-	-	ND
D. variabilis		-	-	ND
A. spp.**	"R. amblyommii"	-	+ (bright)	ND
A. americanum	"R. amblyommii"	-	-	-
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.		-	-	ND
A. spp.		-	-	ND
A. spp.		-	-	ND

Table 2.3 Continued.

Tick species ID	Rickettsia species ID	Tularemia ~400bp	Borrelia Fla 350bp,600bp	P.Mtn. Ehr
A. spp.		-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	smear	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.		-	-	ND
A. spp.		-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.	"R. amblyommii"	-	-	ND
A. spp.		-	-	ND
A. americanum		-	-	-
A. spp.		-	-	ND
R. sanguineus		-	-	ND
A. americanum		-	-	-
A. americanum		-	-	-
A. maculatum		-	-	-
A. maculatum	"R. amblyommii"	-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		+	-	POS
A. maculatum		-	-	-
A. americanum		-	+	ND
Ixodes scapularis		-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	ND
Ixodes scapularis	Ix.scapularis symbiont	-	-	ND
Ixodes scapularis		-	-	ND
Ixodes scapularis		-	-	ND
Ixodes scapularis		-	-	ND
Ixodes scapularis		-	-	ND
A. americanum		-	-	ND
A. maculatum		-	-	-
A. americanum	"R. amblyommii"	-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	ND
Ixodes scapularis	Ix.scapularis symbiont	-	-	ND

Table 2.3 Continued.

Tick species ID	Rickettsia species ID	Tularemia ~400bp	Borrelia Fla 350bp,600bp	P.Mtn. Ehr
A. americanum	"R. amblyommii"	-	-	ND
A. americanum		-	-	ND
Ixodes scapularis	R.cooleyi-like	-	-	ND
Ixodes scapularis	R.cooleyi-like / Ix.scapularis symbiont	-	-	ND
A. americanum		-	-	ND
Ixodes scapularis		-	-	ND
A. maculatum		-	-	-
A. americanum		-	-	-
Ixodes scapularis		-	-	ND
A. americanum		-	-	ND
A. americanum	"R. amblyommii"	-	-	ND
A. americanum	"R. amblyommii"	-	-	ND
A. americanum		-	-	ND
A. americanum	"R. amblyommii"	-	-	ND
A. americanum	"R. amblyommii"	-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	ND
A. americanum		-	-	-
A. americanum	"R. amblyommii"	-	-	-
A. americanum		-	-	ND
Ixodes scapularis		-	-	ND
A. americanum		-	-	ND
Ixodes scapularis		-	-	ND
A. americanum		-	-	ND
Ixodes scapularis		-	-	ND
A. americanum	"R. amblyommii"	-	-	ND
A. americanum		-	-	ND
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-

Table 2.3 Continued.

Tick species ID	Rickettsia species ID	Tularemia ~400bp	Borrelia Fla 350bp,600bp	P.Mtn. Ehr
A. maculatum		-	-	-
A. maculatum		-	-	-
A. maculatum		-	-	-
A. americanum		-	-	-
A. maculatum		-	-	-

** *Borrelia lonestari* sequenced from this specimen

Table 2.4. GenBank accession numbers and associated organisms from ixodid ticks collected in southeast Texas in 2005-2006.

Submitted Sequence Name	GenBank Accession Number	Associated Organism
keith3-4_17kd	EF689727	*Rickettsia canadensis*
keith12_17kd	EF689728	Unnamed Rickettsia from P.D. Teel colony at TAMU
keith12_rompa_70-701	EF689729	Unnamed Rickettsia from P.D. Teel colony at TAMU
keith51_17kd	EF689730	*R. amblyommii*
keith51_rompa_70-701	EF689731	*R. amblyommii*
keith116_17kd	EF689732	*R. amblyommii*
keith116_rompa_70-701	EF689733	*R. amblyommii*
keith125_17kdr1	EF689734	*I. scapularis* symbiont / *R. midichlorii*
keith125_rompa_70-701	EF689735	*I. scapularis* symbiont / *R. midichlorii*
keith136_17kdr1	EF689736	*R. cooleyi*
keith136_rompa_70-701	EF689737	*I. scapularis* symbiont
keith140_17kd	EF689738	*R. cooleyi*
keith141_17kd	EF689739	*R. cooleyi*
keith141_rompa_70-701	EF689740	*R. cooleyi*
keith119_Ehr3CS-inte	EF689741	Panola Mtn. *Ehrlichia*
keith76_fla-internal	EF689743	*Francisella tularensis*
keith119_EhrCS754-12	EF689742	*Borrelia lonestari*

Identification of Agents from Tissue Samples

Microagglutination

There were 109 feral animals tested by MA. There were 59 feline and 50 canine sera samples. All samples tested for *F. tularensis* using MA methods returned negative results (Fig. 2.1).

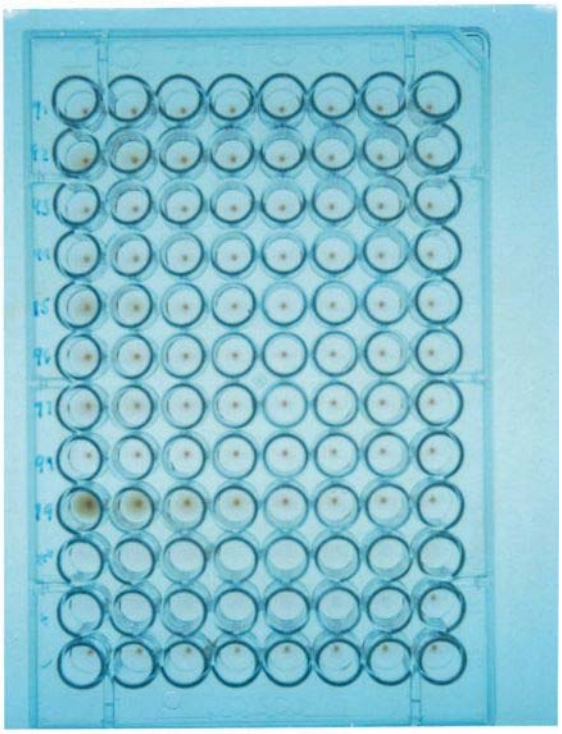


Figure 2.1 Results of microagglutination (MA) assay for antibodies to *Francisella tularensis* from canine and feline samples from Harris County, Texas. Samples 91-99 are in the first nine rows. Rows 10-11 are positive controls and row 12 is negative control. All MA results were negative.

ALISA

F. tularensis was identified from a cat using the ALISA method. Type B tularemia was isolated from this sample at AFRL. These findings were reported by Vivekananda and Kiel (2006).

Discussion

Tick Collections

Ticks were collected from rural, suburban, and urban sites in southeast Texas during 2005 and 2006. The intent of the collections was to attempt to isolate and identify to *F. tularensis* subspecies in host animals and arthropods in southeast Texas, and assess the risk of introduction and spread of tularemia based on vector/host ecology. The rationale was two-fold. First, there is a need to understand the basic ecology of tularemia in southeast Texas. Second, baseline infection rates and identification of hosts, vectors, and pathogen (subspecies) are essential to interpreting positive environmental samples for a pathogen, for example, the tularemia positive BioWatch air samples.

Tick density, as a function of the number of ticks caught per trap, was highest in the rural areas of Sam Houston National Forest and Huntsville State Park (14.31) and lowest in urban areas of Houston (0.10). The suburban site had an intermediate tick density (0.18), but the highest species diversity with four species present. The lowest species diversity was found in the rural site, where *A. americanum* dominated. Species diversity in the urban site was predominantly *A. maculatum*. These results provide an interesting first glance into the host/vector/pathogen interaction of different landscapes,

and certainly deserve more study. However, these results have limited interpretability. Since the purpose of this survey was to identify sources and types of tularemia in the study area, and not a comprehensive and exhaustive tick survey, the data provide an incomplete picture of both density and diversity. The species diversity and density would likely have been very different if surveys had been conducted in all locations year-round for the two-year period. For example, no *I. scapularis* were collected from the rural sites, but surveys were conducted in June and August. *I. scapularis* adults in southern part of the U.S. are not active during this time of year and typically are not found except in fall and early spring (Mackay and Foil 2005).

Screening Ticks for Pathogens

In the two years of gathering data on tularemia, only two samples were positive for *F. tularensis*. Blood (serum) from one cat collected at HCAC in 2005 was positive for type B tularemia. This information was published as part of the validation of the ALISA protocol (Vivekananda and Kiel 2006). One adult female *A. maculatum* was positive for an organism closely related to the causative agent of tularemia. The sequence data (GenBank accession number EF689743) most closely matched *F. tularensis novicida* with a 92% alignment. This was followed by a 91% match with *F. tularensis tularensis*, the most virulent subspecies of *F. tularensis*.

In addition to the *F. tularensis* positive sample, many ticks returned positive results for either pathogenic or endosymbiotic (potentially pathogenic) organisms. The significance of the high percentage of *R. amblyommii* infected ticks is unclear but

warrants further investigation. Coinfection was found in *A. americanum, A. maculatum,* and *Ix. scapularis*. The significance of this finding is also unclear but warrants further field and laboratory investigations to see if there is a relationship between coinfection and increased pathogenicity of one or both organisms involved.

CHAPTER III

POTENTIAL DISTRIBUTION OF *FRANCISELLA TULARENSIS* IN SOUTHEAST TEXAS BASED ON HABITAT CAPABILITY FOR THE LONE STAR TICK, *AMBLYOMMA AMERICANUM*

Zoonotic pathogens are associated with reservoirs, vectors, and susceptible hosts. Diversity and quantity of these epidemiological components are associated with specific habitat types (Semtner and Hair 1973, Patrick and Hair 1978, Diffendorfer et al. 1995). Therefore, habitat types vary in their quantity and diversity of pathogens. *Francisella tularensis*, the causative agent of tularemia, is transmitted in the southeastern U.S. most commonly by the infective bite of hard ticks. The primary vector in the southeastern U.S. is the lone star tick, *Amblyomma americanum* (L.). Ticks become infected by feeding on infective hosts (Hopla 1953). The most important reservoir hosts for tularemia in the U.S. are rodents and lagomorphs (Hopla 1974). Therefore, these reservoir hosts must be present for the pathogen to persist in an area. In addition to reservoir hosts, the risk for tick-borne disease is impacted by amplifying hosts. The white-tailed deer, *Odocoileus virginianus*, is the most important amplifying host for *A. americanum* (Hair and Bowman 1986).

Hosts are necessary to sustain a tick population in a particular habitat but are not the limiting factor for the distribution of a particular species (Randolph 2000). Most ticks spend a relatively short amount of time feeding on hosts. The vast majority of the life cycle is the off-host phase. As with most arthropods, desiccation is the greatest

cause of mortality to tick populations (Needham and Teel 1991). Therefore, it is the combination of host availability (supports the population) and off-host habitat (limits the population) that balances the overall tick population in an area. Models have been developed which take into account these host and habitat variables that influence tick populations.

Remotely sensed (RS) data and geographic information systems (GIS) are often employed for use in epidemiological studies (Kitron 1998, Rogers and Randolph 2003). These tools help identify areas of potential disease outbreak by mapping locations where vectors are likely to be found (Hugh-Jones et al. 1988, Randolph 2002, Daniel et al. 2004). Risk maps based on tick distribution have been developed for several tick-borne diseases, including Lyme borreliosis and human granulocytic ehrlichiosis in the U.S. (Daniels et al. 1998), and tick-borne encephalitis in Europe (Daniel et al. 1998).

Habitat capability/suitability maps for vectors are one of the most common means of estimating the potential for a particular tick-borne disease. The most common habitat capability maps for ticks in the U.S. focus on *Ixodes scapularis*, the primary vector of Lyme borreliosis in this country (Kitron and Kazmierczak 1997, Guerra et al. 2002, Bunnell et al. 2003). These maps employ various techniques to derive the suitable habitat. The most appropriate input for these models depends on the desired output scale.

At the region or continental level (tens of km and greater), climate may be the most appropriate input. Normalized difference vegetation index (NDVI) is a RS vegetative index that measures greenness of the plant material. NDVI is related to

moisture availability and has been correlated to tick mortality rates (Randolph 2000). NDVI has been used in several tick models as input at the regional level (Kitron and Kazmierczak 1997, Daniel et al. 1998, Estrada-Pena et al. 2004). At the landscape level (a few kilometers), land cover type may be the most appropriate, and at the lowest levels (1 km) biotic interaction may be the most appropriate input for generating habitat suitability maps (Estrada-Pena 2006).

Therefore, my purpose was to create a habitat capability map for the lone star tick, *A. americanum*, in the Houston, TX, area as an indicator of the potential spread of tularemia, and other *A. americanum*-borne pathogens, across the landscape. A habitat capability map for *A. americanum* could be used to predict not only potential areas for tularemia and other pathogens transmitted by this vector, but as a general risk map for areas where one is likely to encounter this common and aggressive species.

Materials and Methods

The data for my model were based on the normalized difference vegetation index (NDVI) from 2002 and a nine category land classification system generated from RS data of southeast TX taken in 2002. The land classification was generated for the Houston-Galveston Area Council (HGAC) and Clean Rivers Program Region as an aid in studying and understanding of water quality in the region (Fig. 3.1). The area covers 13 counties in the Houston/Galveston region and grids were at 30 m^2 resolution. These

data, and their complete description, are available from the HGAC website (Horton 2003). The NDVI data, which were already derived, were made available for GIS projects during coursework at the Spatial Sciences Laboratory at Texas A&M University in 2007.

The habitat capability model used in this study is based on the interpretation of landscape characteristics derived from remotely sensed data, i.e., land classes and NDVI. Assumptions are that different land classes vary in their ability to support tick/host/pathogen populations. These assumptions of species use and movement within different landscape types are well supported in theory (Forman 1995), and with data from the epidemiological components (Semtner and Hair 1973, Patrick and Hair 1978, Anderson et al. 2003, Gaughan and Destefano 2005). Of the nine land classes, only woody land and woody wetland were chosen as good habitat from the land classification (Fig 3.2).

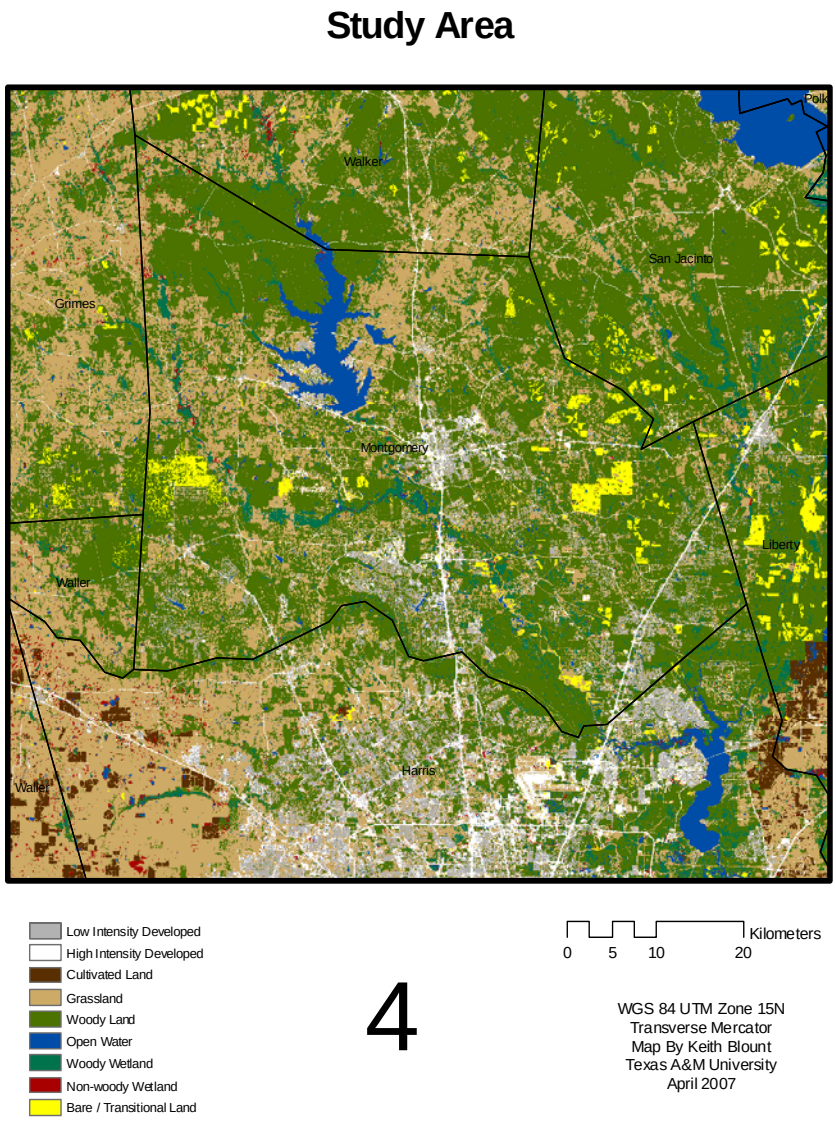


Figure 3.1 Study areas for the habitat capability model of the lone star tick, *Amblyomma americanum*. Figure shows the nine-category land classification for Houston, TX, and areas north.

The NDVI values were derived at a 1 km^2 resolution in 21-day intervals, which were then averaged over one year and reclassified (Fig 3.3). Grids with values equal to or greater than 0.6 were selected as good habitat for *A. americanum* (Fig. 3.4). This value is equal to values in the literature for NDVI (Estrada-Pena 2002) and agrees with aerial photography of the study site also taken in 2002.

Combining good habitat for the hosts based on the land classification, with NDVI values supporting vectors, results in an estimate of the habitat capability for *A. americanum* (Fig. 3.5). This model takes into consideration host utilization of the habitat, yet is constrained to areas only where *A. americanum* survival is most likely based on off-host ecology.

Indicator kriging has been used to estimate the risk of vector-borne disease transmission (Beroll et al. 2007). I used this method to estimate the probability of finding good *A. americanum* habitat in the study area (Fig. 3.6). This interpolation method produces a probability map based on surpassing a preset threshold for a hazard (Krivoruchko 2001). In this case, the threshold was one since the input data for the krige was the capability map, which was converted to a binary (0 = bad habitat, 1 = good habitat).

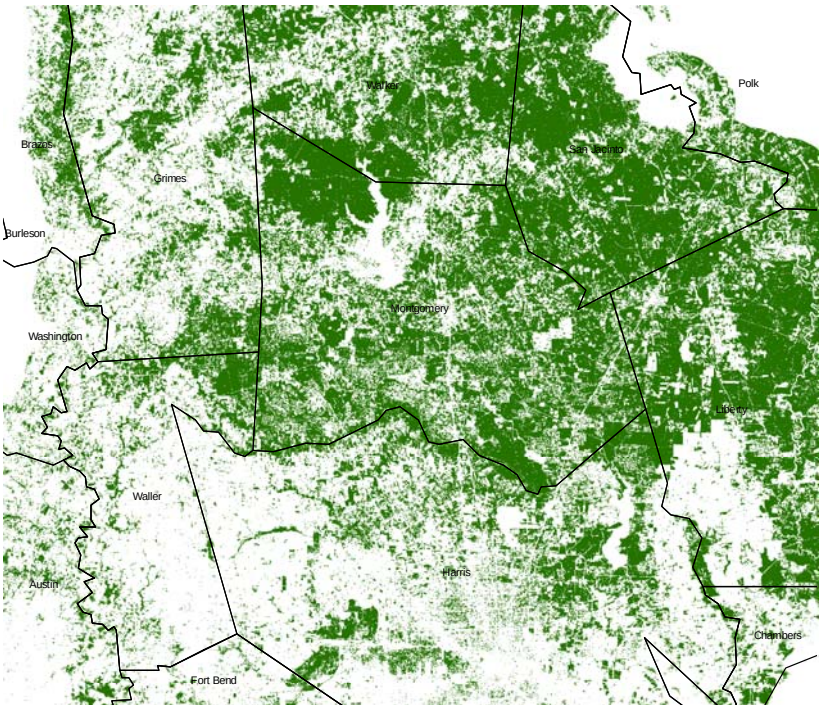


Figure 3.2. Optimal habitat for *A. americanum* in southeast Texas based on a land classification that combines woody land and woody wetland**.**

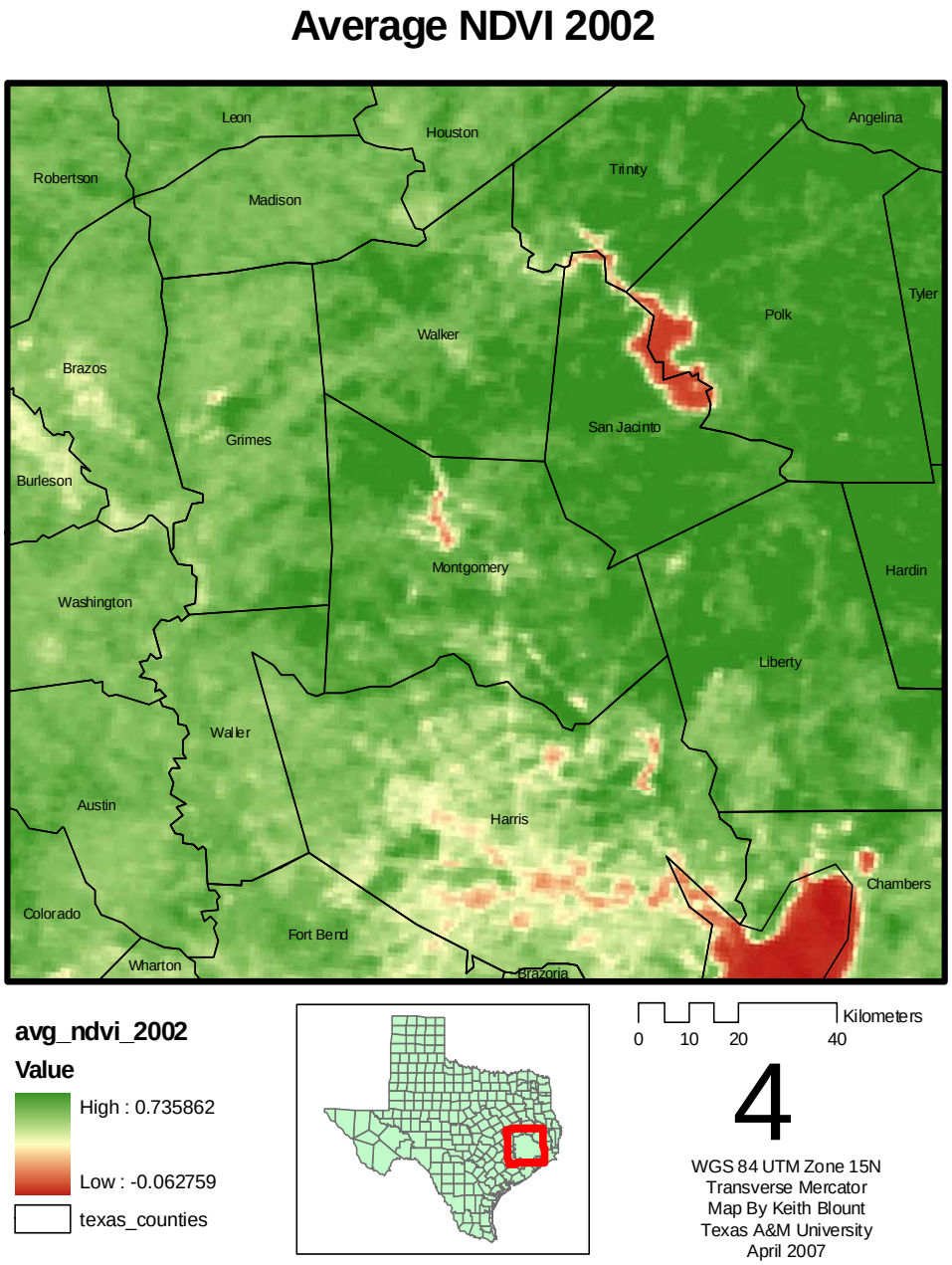


Figure 3.3. Average NDVI of *A. americanum* study area in southeast Texas for 2002. Areas shaded in darker green indicate vegetation and available moisture which is necessary for *A. americanum* survival**.**

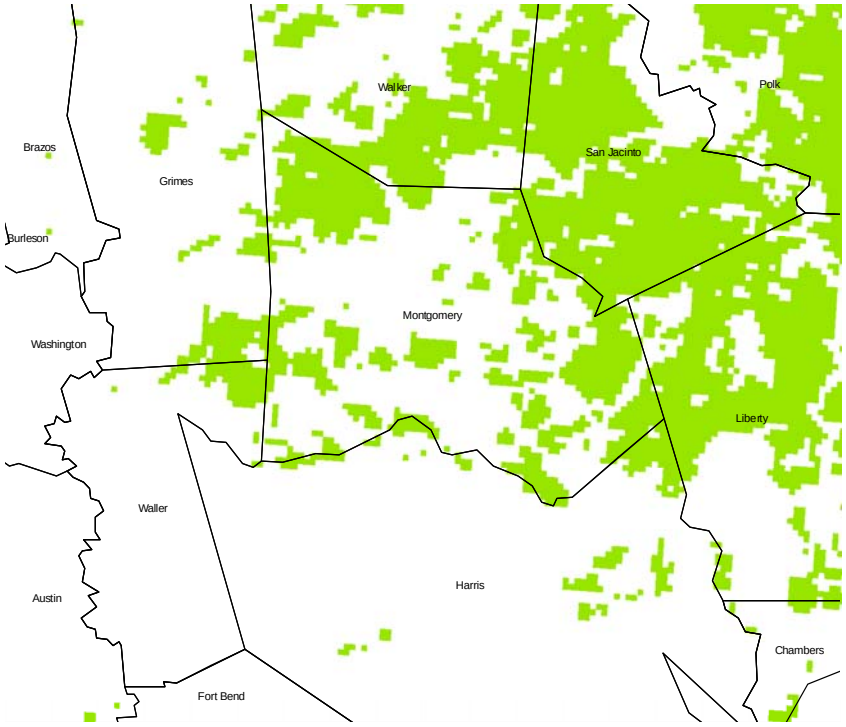


Figure 3.4. Optimal habitat for *A. americanum* in southeast Texas based on NDVI. The areas shaded in green indicate NDVI values greater than or equal to 0.6**.**

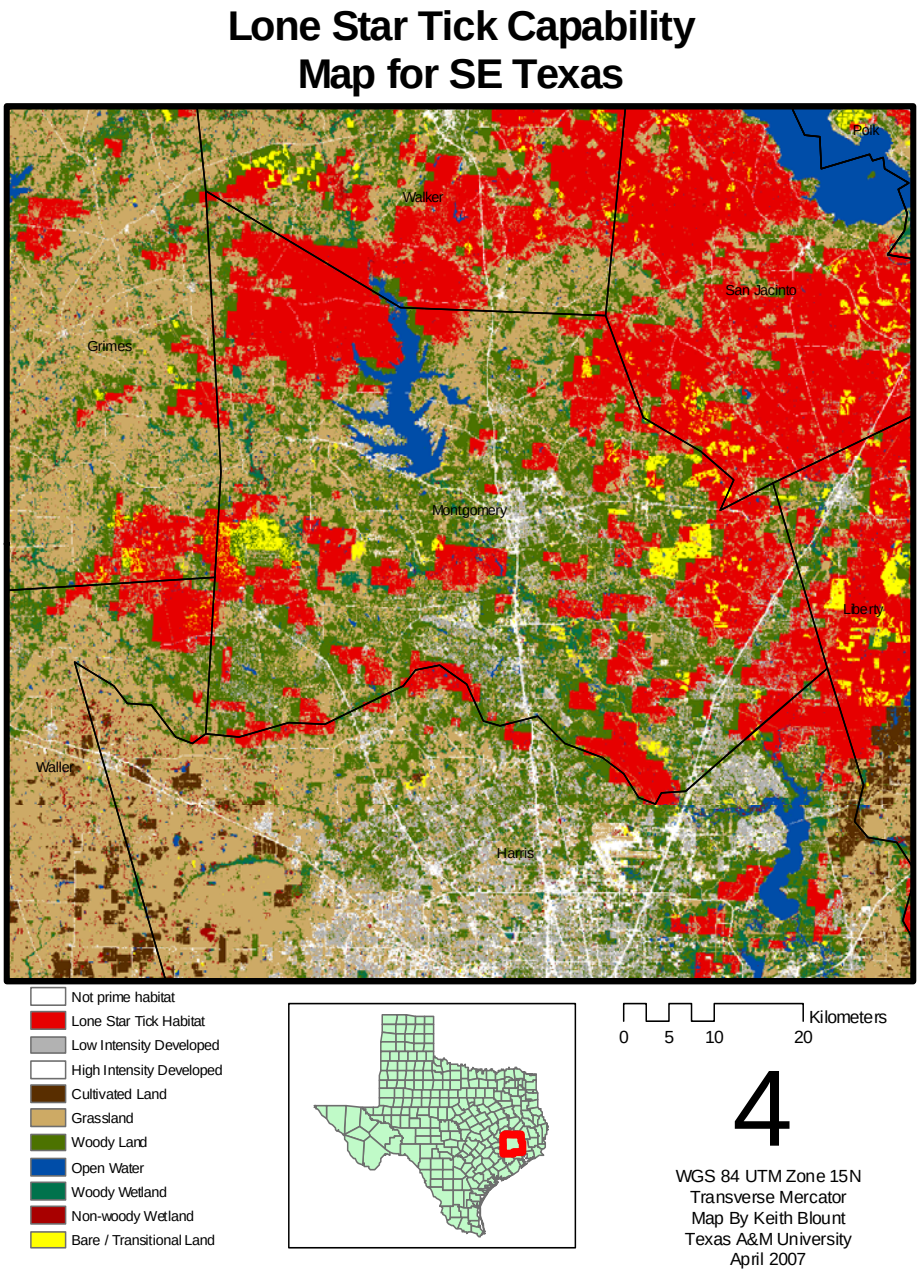


Figure 3.5. The habitat capability map for *A. americanum* in southeast Texas. Areas shaded in red indicate areas where *A. americanum* could likely be found based on both host animal habitat and off-host ecology**.**

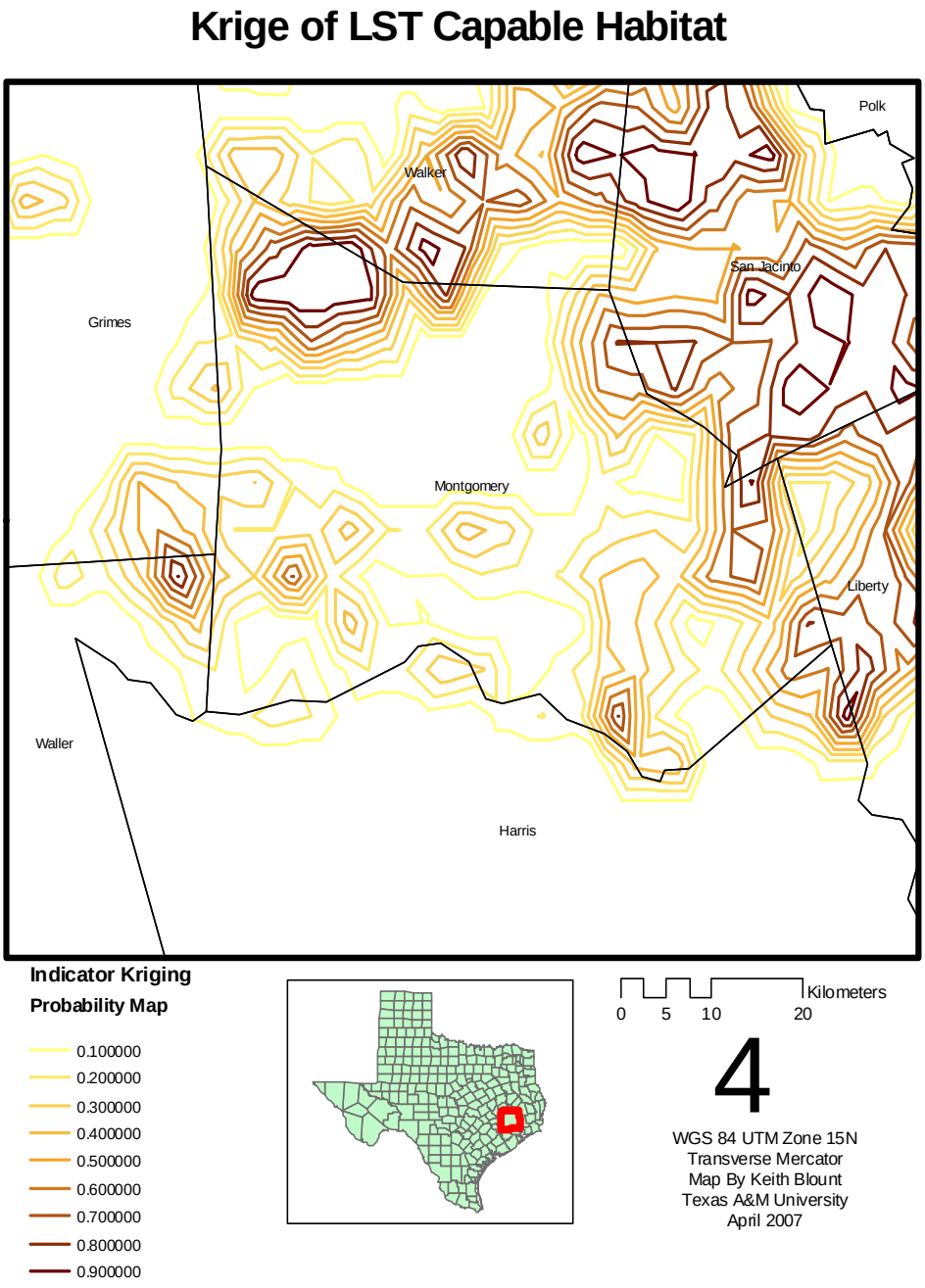


Figure 3.6. Krige of habitat capability model for *A. americanum* in southeast Texas. Concentric darkening lines show increasing probability of identifying good habitat for *A. americanum*.

Results and Discussion

The lone star tick habitat capability model generated areas likely to support high populations of *A. americanum* in the Houston area. The suitable habitat based on land classification only (Fig. 3.2) was considerably larger than the overall area generated in the final capability map (Fig. 3.5). Even though land class is an appropriate input for estimates of tick distributions at the landscape scale (Estrada-Pena 2006), I believe this input used alone would over-estimate the habitat that could sustain *A. americanum* populations. NDVI is often used as the input for tick distributions at scales larger than the landscape (Randolph 2000) because of the relationship of NDVI to climate. However, these data could be used as the limiting factor in tick distribution, as it is for this project. This not only incorporates valuable environmental data, it allows for limits in population distribution based on the off-host ecology of the tick.

As with any model, there are assumptions and limitations. The NDVI and land classification used in this model are five years old. Given the growth of the Houston/Galveston area, these data are likely already outdated. Urban sprawl, the rate of growth of suburbia, and ever changing land utilization within this landscape necessitate continual update of data and models.

The land classification and NDVI data used for this habitat capability are also at different scales. The land class is at 30 m resolution, whereas the NDVI is at 1 km resolution. Had the NDVI grid matched the land classification in resolution (30 m), the final map would likely have looked slightly different. This is especially true in the suburban areas where good habitat (mainly woody land) is present but more fragmented

and smaller in size than that in rural areas. The difference in cell size resulted in a slight loss of what would likely be suitable habitat, based on results of field data from tick trapping (Chapter II). If however, a capability map covering a larger area were desired, then increasing the land classification to 1 km resolution would be more appropriate than lowering the NDVI resolution to 30 m.

These model results need to be validated through extensive sampling of ixodid ticks in all the land classes within the study area. Correlations of the habitat capability model with results of extensive arthropod surveillance and epidemiological data for *F. tularensis* in human and host mammals will help validate this model and provide credence to the methodology as well.

The probability map generated by the krige of the habitat capability map provides additional information about the potential *A. americanum* habitat distribution in the study area. Like the habitat capability map, the results of the probability map need to be verified by extensive field sampling. One of the limits of the probability map is that it may focus too heavily on the center of the suitable habitat areas and not enough on the borders. As some species prefer edges of habitat as opposed to the center of a habitat, this particular model may not allow for influence of ecotones.

Pathogens like *F. tularensis* are frequently found at very low levels in nature. Determining the prevalence of a pathogen that is rarely encountered is costly in time, money, and manpower. Products like this habitat capability model can be valuable tools to aid in tick surveillance. They can also be useful surrogates when identification of the actual pathogen is prohibitive.

CHAPTER IV

MODELLING THE MOVEMENT AND PERSISTENCE OF *FRANCISELLA TULARENSIS* IN SOUTHEAST TEXAS

Tularemia is one of the most complex zoonotic diseases known. Over 250 vertebrates and invertebrates have been associated with the bacterial causative agent, *Francisella tularensis*. The bacterium remains in the environment at very low levels. The disease is not common in humans now, with fewer than 200 cases per year on average in the U.S., compared to greater than 1,000 cases per year in the 1940s. Epizootics are seldom seen due to the small size and secretive nature of hosts. Therefore, field collections of *F. tularensis* are rare and data are sparse. Due to declining numbers of clinical cases and the recent constraints on transporting the pathogen and working with it in the laboratory, it is unlikely there will be extensive field studies undertaken to solve many of the basic ecological questions.

Few additional details about the ecology of the disease have been elucidated in the last 30 years, even though substantial research on identifying effective clinical treatments and molecular characterization of this pathogen are currently underway. The increasing gap between our knowledge of the molecular biology of *F. tularensis* and our understanding of this agent's ecology recently grew wider with the publication of the complete genomic sequence of *F. tularensis* (Larsson et al. 2005). By contrast, we still have little understanding of where, how, and at what levels, *F. tularensis* maintains itself

in nature and what factors affect the transmission dynamics among hosts and vectors, as well as human risks of infection.

Interest in tularemia increased in the late 1990s as the United States began to realize the number of nations and other entities that possessed or might possess biological weapons or the knowledge of BW production. As a result, the Department of Homeland Security, in conjunction with the Centers for Disease Control and Prevention (CDC) and Environmental Protection Agency promulgated a national air monitoring program called BioWatch which collects air samples in large metropolitan areas to test for select BW agents. In 2003, samples collected in Houston, TX, indicated airborne *Francisella tularensis* (Houston 2003).

BioWatch air samples continued to indicate air-borne *Francisella* through 2004. Initial investigations by local, state, and federal authorities could not identify with certainty a source for the positive air samples based on data from rodent surveys. Confirmatory testing in 2004 revealed the initial positive air sample was likely a new strain of *F. tularensis* or non-pathogenic strain of *Francisella philomiragia* that can be present in many semi-aquatic and terrestrial environments (Barns et al. 2005).

The positive air samples in Houston (believed to be positive at the time) did more than indicate the presence of an airborne pathogen. It brought to the forefront a fundamental problem with the BioWatch surveillance program: interpretation of positive environmental results from an area with little to no background data, and no understanding of the ecology or epidemiology of the disease in the urban environment. This begs the question of what is the appropriate response to a positive air sample when

there is no understanding of background levels of the pathogen in the animal/vector population or the environment. Severity of disease and likelihood of exposure are fundamental to the risk assessment process, but cannot be accurately estimated without knowing the source of exposure and its virulence. If an air sample is positive for a virulent form of a pathogen, is there any way to know whether a pathogen might spread and where it is likely to be in 24 hours, 24 days, or 24 months after it is detected? Finding answers to these types of questions, given the current limitations to research, is a daunting but important task.

Individual-based models are useful tools for estimating interactions of complex ecological systems like tularemia (DeAngelis and Mooij 2005). Simulation models often are used to project future dynamics of populations of species and to estimate risk of population extinction. However, the simulation models developed for tularemia have been clinically based, and typically provide guidelines for personnel staffing or treatment regimes (Hupert et al. 2002, Burr et al. 2007). Individual-based simulation models of the lone star tick, the primary arthropod vector of *F. tularensis* in the southern U.S., were developed for integrated pest management of this arthropod (Mount and Haile 1987, Mount et al. 1993), but these lack spatial applications needed to address the complex systems associated with diseases vectored by this tick species.

The purpose of this chapter is to describe the development, evaluation, and application of a simulation model of the movement and persistence of *F. tularensis*, the causative agent of tularemia, introduced into different landscapes in Houston/southeast Texas. The model is a spatially-explicit, agent-based, stochastic simulation model. The

model is programmed in VB.NET© (Microsoft, 2003) and results are viewed using the ArcView (ESRI, 2005) GIS platform.

Materials and Methods

Overview of the Model

Spatial and temporal resolution and extent of the model system:

The model system represents either a 0.36 km^2 rural/urban landscape (small grid) divided into 400 30m by 30m cells, or a 9 km^2 rural/urban landscape (big grid) divided into 10,000 30m by 30m cells. Simulations were run for one to three years using a daily time step. Simulations took place in grids representing real landscapes in southeast Texas (Fig. 4.1).

The model is composed of the primary ecological and epidemiological components of tularemia in nature: the pathogen, vector, and hosts (both pathogen and vector hosts). This model was written specifically for *F. tularensis*, but could be modified for many different vector-borne or zoonotic diseases. The generic variables of the model are following with specific counterparts in parenthesis. The entities in the model are: pathogen, *F. tularensis* (referred to as Tula in the model); arthropod vector, *Amblyomma americanum* (referred to as Tick in the model); reservoir, small mammals (referred to as Smam in the model); and amplifying host, white-tailed deer, *Odocoileus virginianus* (referred to as Deer in the model).

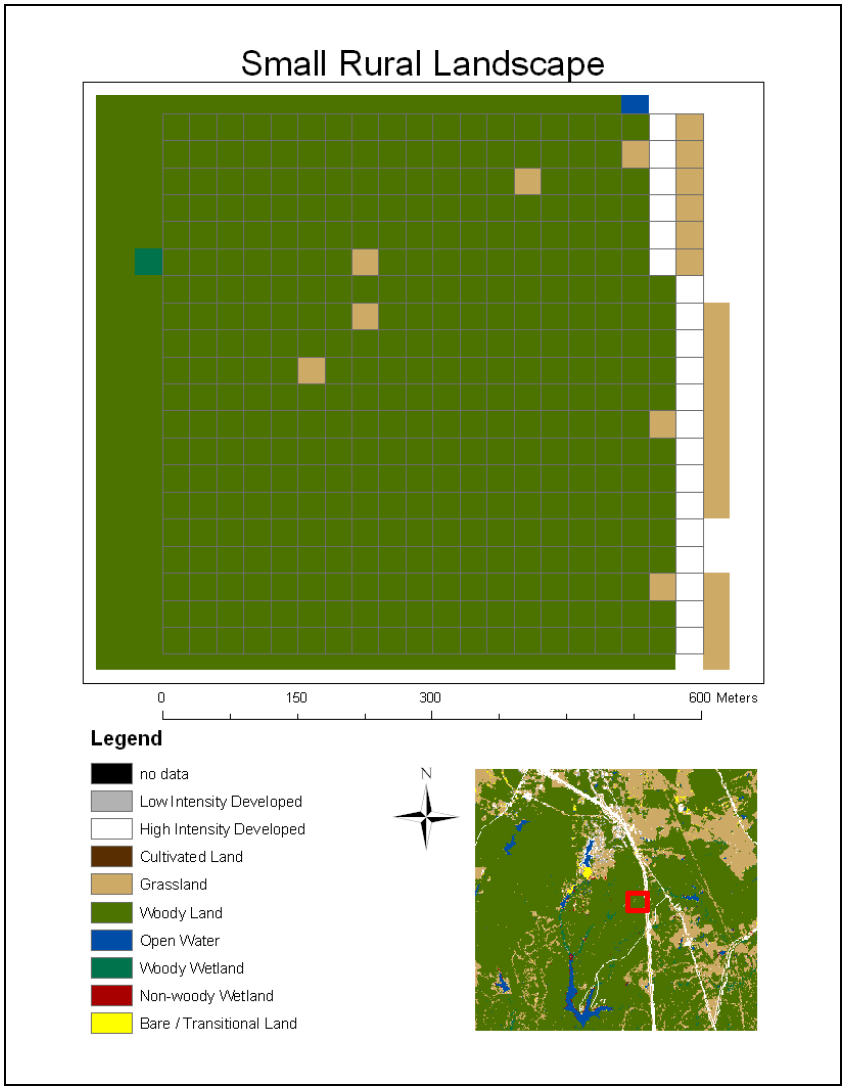


Figure 4.1. Small rural landscape grid used for modelling simulations of *Francisella tularensis*.

Attributes of cells in the landscape grid include 12 static descriptors indicating spatial position and land classification (Table 4.1), 11 state variables representing the current levels of tularemia, ticks, small mammals, and deer (Table 4.2), and 7 aggregated variables representing the current presence (in any form) or absence of tularemia and the history of tularemia within each landscape cell (Table 4.3). Cells within the grids are based on a land classification for the year 2000 as previously described (Chapter II).

Table 4.1. List of the 12 static landscape cell descriptors.

ID number
Latitude of midpoint
Longitude of midpoint
ID numbers of each of the 8 adjacent cells, identified as northern neighbor, northeastern neighbor, eastern neighbor, etc.
Land classification indicating the type of land cover within the cell

Table 4.2. List of the 11 state variables representing the current levels (arbitrary units) of tularemia, ticks, small mammals, and deer within each landscape cell.

Tularemia in the abiotic environment
Non-infected (susceptible) ticks
Non-infected (susceptible) ticks in diapause
Infected ticks
Infected ticks in diapause
Non-infected (susceptible) small mammals
Infected small mammals
Recovered (immune) small mammals
Deer without ticks
Deer carrying uninfected ticks
Deer carrying infected ticks

Table 4.3. List of the 7 aggregate variables representing the current presence (in any form) or absence of tularemia and the history of tularemia within each landscape cell.

Tularemia presence (in any form) or absence
Total number of time steps that cell has been infected during simulation
Total number of times the cell has been newly-infected during simulation
Time since last new infection of tularemia (in any form)
Time since last new infection via ticks carried by deer
Time since last new infection via ticks carried by small mammals
Time since last new infection via small mammals

The sequence of operations during the execution of a simulation is summarized in Table 4.4.

Table 4.4. Summary of the sequence of operations during the execution of a simulation.

Read landscape data and create initial instances of classes
Record initial conditions and create maps
Begin calculations for this time step (day)
Update day of year and other counters
Move ticks into or out of diapause if appropriate
Move Smam and Deer to adjacent landscape cells
Calculate Tula, Tick, Smam, and Deer decay within landscape cells
Calculate disease transmission and loss within landscape cells
Record current conditions and create maps
End simulation

Entities in the model have two basic functions: movement and persistence. Movement is a probability function of type of entity. For example, in most of the simulations, deer have a higher probability of moving to an adjacent cell than small mammals, which, in turn, have a higher probability of moving than unattached ticks (essentially zero).

Persistence of an entity is a function of decay. Decay rates are habitat dependant and based on a nine category land classification. Decay rates for each epidemiological component were adjusted between 0 and 1 based on available literature, expert opinion, and field data for each of the nine land classes in the classification scheme. The lower the decay rate for an entity, the more likely it is to persist in a given habitat type and landscape. Entities in high intensity developed land have a very high decay rates, whereas entities in woody land have low decay rates. A simple but effective way to envision the decay rates for entities in land classes is to imagine the outcome for a given entity in unsuitable high intensity developed land versus good habitat like woodlands – small mammals, white-tailed deer, and lone star ticks do quite well in most forest ecosystems, but not on heavily traveled highways.

Unlike small mammals, deer are not important reservoirs for *F. tularensis* (Burgdorfer et al. 1974, Cooney and Burgdorfer 1974); however, they are very important for regulating tick numbers (Hair and Bowman 1986b, Ginsberg et al. 2002). During each time step, deer and small mammals move to an adjacent cell based on their movement probability. Deer function in the simulation by moving uninfected and infected ticks throughout the landscape. Small mammals function in the simulation by

moving uninfected and infected ticks throughout the landscape, and as a source of infection to uninfected ticks. Ticks (larvae, nymphs, and adults) function in the simulation as vectors of the pathogen, and as a source for new infections each spring due to the pathogen overwintering in the vector (Hopla 1953, 1955). *A. americanum* life stages overwinter at latitudes encompassing southeast Texas as fed and unfed nymphs, and unfed adults (Semtner 1973). These rules are based on the ecology and epidemiology of tularemia (Hopla 1974, Dennis 1998).

If ticks are present in a cell in which deer or small mammals move, they attach. All feeding stages of lone star ticks parasitize white-tailed deer (Hair and Bowman 1986b, Durden et al. 1991) and immature lone star ticks parasitize small mammals (Cooney and Burgdorfer 1974, Cooney et al. 2005). If the animals were already carrying ticks when they moved to an adjacent cell, then the new cell becomes infested with ticks due to ticks dropping off infested animals. Ticks can either be uninfected or infected with *F. tularensis*. If uninfected ticks attach to an animal that is not infected with *F. tularensis*, then the ticks remain uninfected and simply use the animal as a source of nutrition and movement. If, however, the tick is uninfected and attaches to an infected small mammal, the tick becomes a vector of *F. tularensis* and transmits the infection to all susceptible hosts (uninfected or recovered small mammals). Susceptible small mammals are mammals that are not infected, or have been infected but recovered, i.e., they no longer are exposed to vectors and have gone through a recovery period (10 days).

Seasonality in the simulated landscape is represented by a decline in tick activity in the late fall/winter. Reduction of the tick population in the model reflects the seasonality of *A. americanum* driven by photoperiod (Pound et al. 1993). Even though photoperiod is the predominant force that regulates diapause in ticks, not all individuals in a population abide by this stimulus. At latitudes encompassing southeast Texas, *A. americanum* can be found on host animals in low numbers as early as January if environmental conditions are favorable (Teel et al. 1990). Therefore, interpretation of model results must be taken in the context of how the model was parameterized. Absence of *F. tularensis* within the model over the winter does not necessarily equate to no risk of acquiring infection from *F. tularensis* or other tick-borne pathogens. Ticks emerge from diapause in early spring, seek hosts, and the cycle continues (Hair and Bowman 1986a). An overview of the sequence of operations is shown in Fig. 4.2.

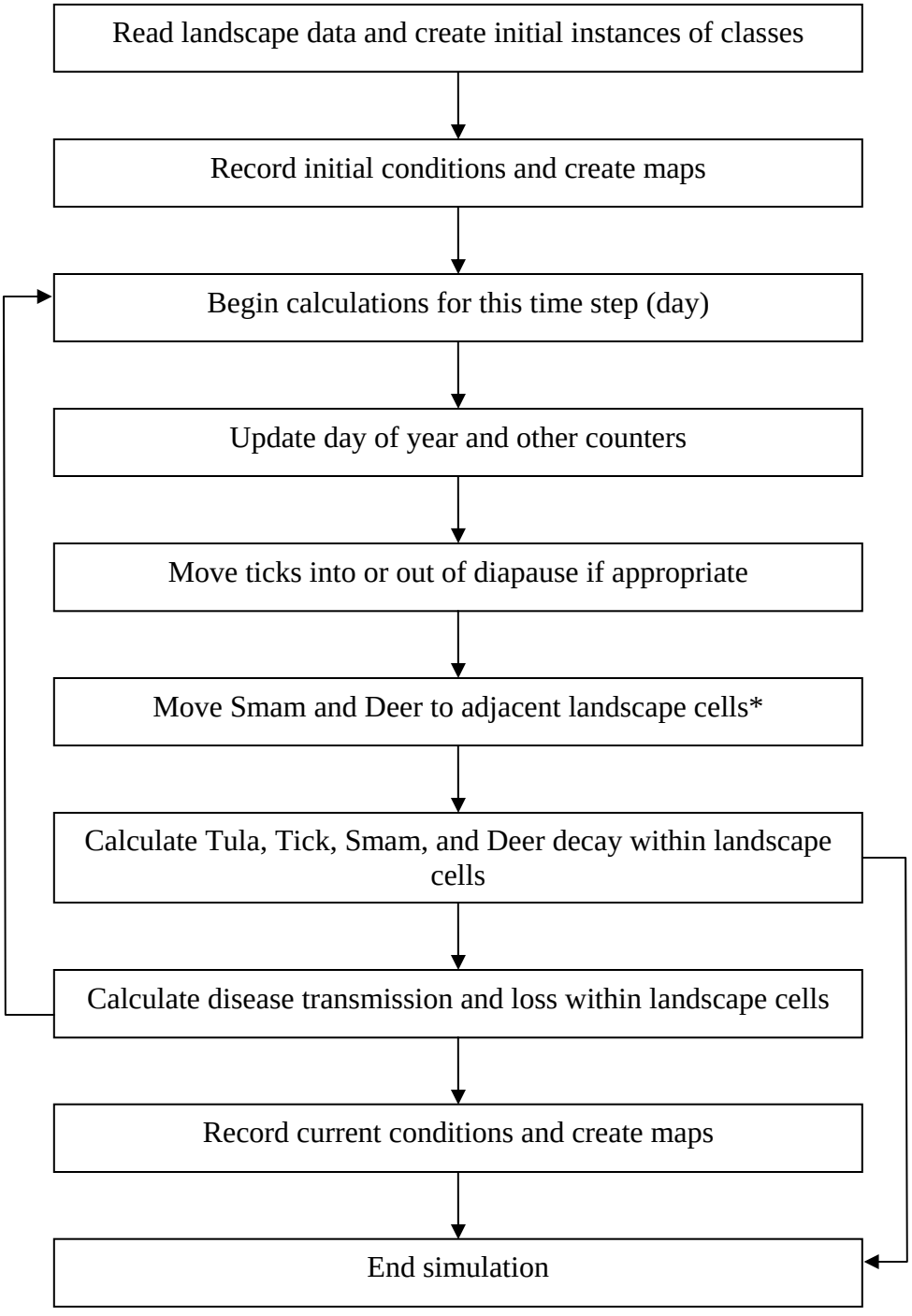


Figure 4.2. Flow chart of the sequence of operations for tularemia dispersal model in southeast Texas. * denotes stochastic process.

Model Description

Initialization

The model is initialized by specifying the day of year to start the simulation and the beginning levels of the state variables in each landscape cell. The simulation can be initialized in either a single cell or all cells. The purpose of point versus system-wide initialization is to simulate a single infected entity being introduced into an area, which is more realistic for a naturally occurring epidemiological event, versus total system infection, which is the likely scenario for an aerosol release of a biological agent in a bioterrorism event.

Initialization can also be varied by introducing *F. tularensis* through a single entity, or a combination of entities. For example, the simulation can be initialized by introducing an infected small mammal into a single cell, or by introducing *F. tularensis* infected ticks into all cells. This permits evaluation of different epidemiological situations. For example, will disease progress through an area differently if introduced in an arthropod vector versus an infected mammal?

Input and Output

Input to the model includes the land classifications for each landscape cell. These data were obtained free of charge from the Harris-Galveston Area Council website (Horton 2003). The land classification data were assigned to each landscape cell by spatially joining equal sized grid cells (30m by 30m) overlaid on the landscape polygon and capturing the associated grid data.

Output from the model includes a time series of maps indicating the daily changes in the spatial distributions of the presence of tularemia (in any form, an aggregated variable) and of each of the 12 state variables (Table 4.1) across the simulated landscape. Output also includes system-level descriptors summarizing the spatial and temporal dynamics of tularemia during the simulation (Table 4.5).

Table 4.5. Model output representing system-level descriptors summarizing the spatial and temporal dynamics of tularemia during the simulation.

Proportion of landscape cells infected with *F. tularensis*
Proportion of Woody Land landscape cells infected with *F. tularensis*
Proportion of Grassland landscape cells infected with *F. tularensis*
Proportion of Etc. landscape cells infected with *F. tularensis*
Total amount of time during simulation landscape was infected with *F. tularensis*
Total amount of time during simulation Forest cells were infected with *F. tularensis*
Total amount of time during simulation Grassland cells were infected with *F. tularensis*
Total amount of time during simulation Etc. landscape cells were infected with *F. tularensis*

Tick populations are regulated primarily by environmental conditions (Needham and Teel 1991). The lone star tick enters diapause (individuals no longer quest or feed), on average, by the end of October (Sonenshine 1993). Host seeking and feeding in the Houston area begins mid-March (Fleetwood et al. 1984). This overwintering behavior is simulated by induced diapause on day-of-year 304. Tick activity resumes on day-of-year 74 of the simulated year.

Submodels

Read Landscape Data and Create Initial Instances of Classes

Record Initial Conditions and Create Maps

The initial values of the static descriptors (Table 4.1), the state variables (Table 4.2), and the aggregated variables (Table 4.3) in each cell, are written to Excel files. Values of state variables, and the aggregated variable representing tularemia presence (in any form) or absence, are written to data base files linked to shape files loaded into ArcMap.

Move Ticks Into or Out of Diapause if Appropriate

All ticks in the system, including those on small mammals and deer, enter diapause on day-of-year 304 and emerge from diapause on day-of-year 74. Ticks emerge from diapause in the same state that entered diapause with respect to being infected with *F. tularensis* or not.

Move Small Mammals and Deer to Adjacent Landscape Cells

Each day, the small mammals and deer in each cell have probabilities of 0.5 and 1.0, respectively, of moving into an adjacent cell. The cell into which they move is chosen randomly from among the 8 adjacent cells. If a move is made, the level of small mammals, which may be non-infected, infected, or recovered (Table 4.2), or deer, which may be without ticks, carrying uninfected ticks, or carrying infected ticks, in the donor

cell is added to the level in the recipient cell, but if the resulting sum is greater than 1.0, it is reduced to 1.0. The level of small mammals or deer in the donor cell is not reduced.

Calculate Tularemia, Tick, Small Mammal, and Deer Decay within Landscape Cells

The levels of tularemia in the abiotic environment (Tula), ticks (Tick), small mammals (Smam), and deer (Deer) are decreased each day by a proportion that depends on the landscape classification of the cell (Table 4.6). Figure 4.3 shows the user interface of the model and how decay rate levels appear when the model is initiated.

Table 4.6. Model parameters representing decay rates of tularemia in the abiotic environment, and decay rates of ticks, small mammals, and deer, as a function of landscape classification.

	Decay Rate			
Landscape Classification	Abiotic	Tick	Small Mammal	Deer
Low Density Developed	0.97	0.90	0.75	0.80
High Density Developed	0.99	0.99	0.99	0.99
Cultivated Land	0.97	0.95	0.25	0.50
Grassland	0.92	0.50	0.10	0.20
Woody Land	0.95	0.05	0.05	0.05
Open Water	0.95	0.99	0.99	0.99
Woody Wetland	0.85	0.25	0.80	0.10
Non-woody Wetland	0.90	0.90	0.95	0.95
Bare/Transitional Land	0.98	0.95	0.97	0.97

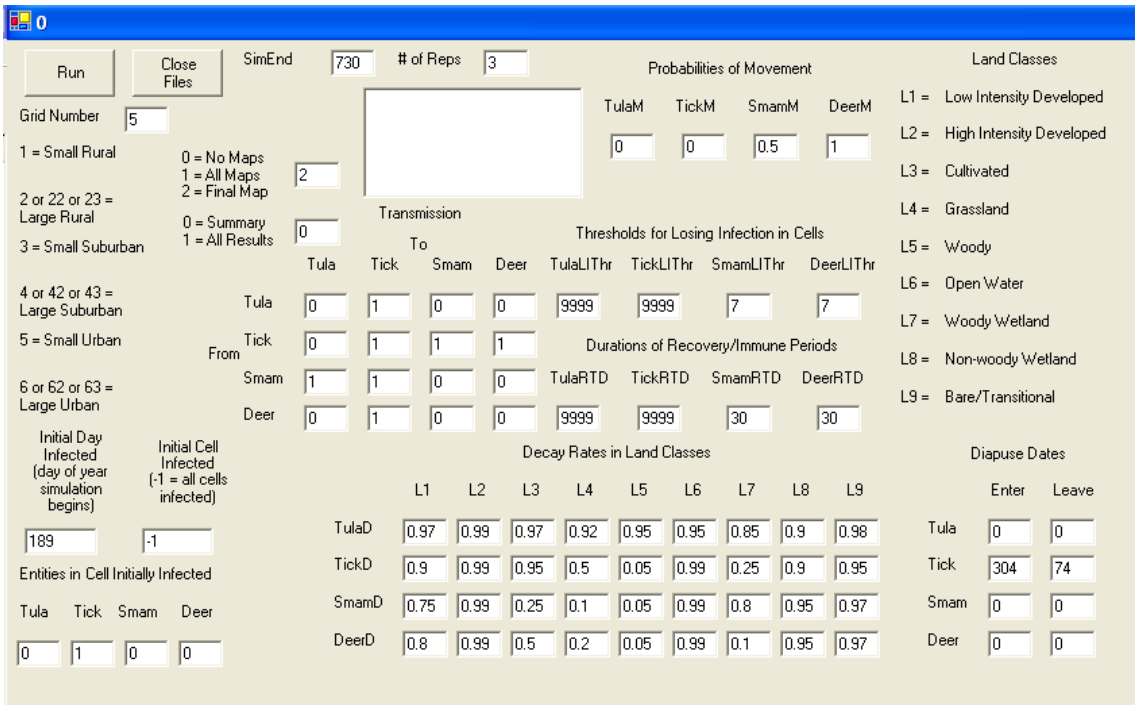


Figure 4.3. User interface of the tularemia model. Tula represents *F. tularensis* in the abiotic environment. Smam represents small mammals. TulaM represents *F. tularensis* movement in the abiotic environment. TickM represents lone star tick movement. SmamM represents small mammal movement. DeerM represents white-tailed deer movement.

Calculate Disease Transmission and Loss within Landscape Cells

First, the infected small mammals and the deer carrying infected ticks that have entered each landscape cell today are identified. Then the transmission of *F. tularensis* among the abiotic environment, ticks, and small mammals is calculated as a function of the appropriate daily *F. tularensis* transmission probabilities (Table 4.7). Then small mammals are transferred from infected to recovered/immune if small mammals within the cell have not been newly infected within the last 7 days. Small mammals are

transferred from recovered/immune to non-infected/susceptible if they have been recovered/immune for 30 consecutive days.

Table 4.7. Model parameters representing daily probabilities of *F. tularensis* transmission within a landscape cell among ticks, small mammals, deer, and the abiotic environment. Parameters were changed for different treatments.

Pathogen Transmission		*From*			
		Abiotic	Tick	Small Mammal	Deer
	Abiotic	0	1	0	0
To	Tick	0	1	1	1
	Small Mammal	1	1	0	0
	Deer	0	1	0	0

Record Current Conditions and Create Maps

The current values of the state variables (Table 4.2), and the aggregated variables (Table 4.3) in each cell, are written to Excel files. Values of state variables, and the aggregated variable representing tularemia presence (in any form) or absence, are written to data base files linked to shape files loaded into ArcMap, as described above.

Results

This model was evaluated for its usefulness at predicting spatial and temporal movement of *F. tularensis*, the pathogen that causes tularemia, across different landscapes.

Comparison

Model results are difficult to compare to events in nature since the simulation results are based on the movement of the pathogen in entities that are rarely sampled, i.e., the model represents epizootics (disease outbreak in an animal population) and available data are based on epidemics (outbreak in human population). Additionally, the system-wide initialization of this model simulates release of an aerosolized pathogen over a large area (entire simulated landscape) – a scenario that has no comparison. Also, the simulation reflects a long standing public health mantra – err on the side of caution. In other words, it is better to overestimate the impact of a disease or event, and be overly prepared, than to underestimate its impact, and suffer significant morbidity or mortality. Recent examples of this concept in public health are found in SARS and avian influenza preparedness (Webby and Webster 2003, Fouchier et al. 2005). This was the approach used in the design and development of this model.

Sensitivity Analysis

Sensitivity analyses for simulations were conducted on changes in the movement probability for small mammals and deer. Even though the probability of movement remains constant throughout a simulation, direction of movement is stochastic. Therefore, changes in movement probability affect overall outcome. Deer in the simulated world, as in the real world, move greater distances than small mammals (Schmidly and Davis 2004). Mean proportion of the system infected, as defined as some

entity within a cell infected with *F. tularensis* at simulation end, is sensitive to animal movement.

Model Results

The four main treatments evaluated landscape type and type of introduction. Simulations were initialized in both rural and urban landscapes, using either point introduction (*F. tularensis* infected tick introduced into a single cell) or system-wide introduction. To evaluate the effect of movement on the persistence of *F. tularensis* in different landscapes, movement probabilities were varied in both deer and small mammals. To evaluate the effect of varying decay rates on movement and persistence of *F. tularensis*, changes were made in land classification decay rate parameters.

Rural landscapes consistently had a higher proportion of infection than urban landscapes, regardless of type of introduction (Fig. 4.4). The mean proportion (and standard deviation) of the system infected for rural system-wide and point introductions were 0.9475 (0.0000) and 0.9458 (0.0014), respectively. The mean proportion of the system infected for urban system-wide and point introductions were 0.8138 (0.0018) and 0.8100 (0.0109), respectively.

System-wide introductions reach equilibrium quicker than point introductions (Fig. 4.5). In the rural landscape, system-wide introduction reaches equilibrium almost immediately, compared to 10 days in the urban landscape. In areas with high movement potential, i.e. rural landscape, total system infected remains unchanged from year to year (Fig. 4.6). Results of simulations for the four primary treatments (rural versus urban)

and introduction (point versus system-wide) are shown in Fig. 4.7. Habitat dominated by woody land remains infected throughout the simulated year, until ticks diapause and infected animals recover.

In both rural and urban environments, reducing dispersal of the pathogen by removing deer or reducing movement of small mammals results in less total infection in the system (Fig. 4.8). The same effect is achieved by increasing the decay rate of the environment (Fig. 4.9).

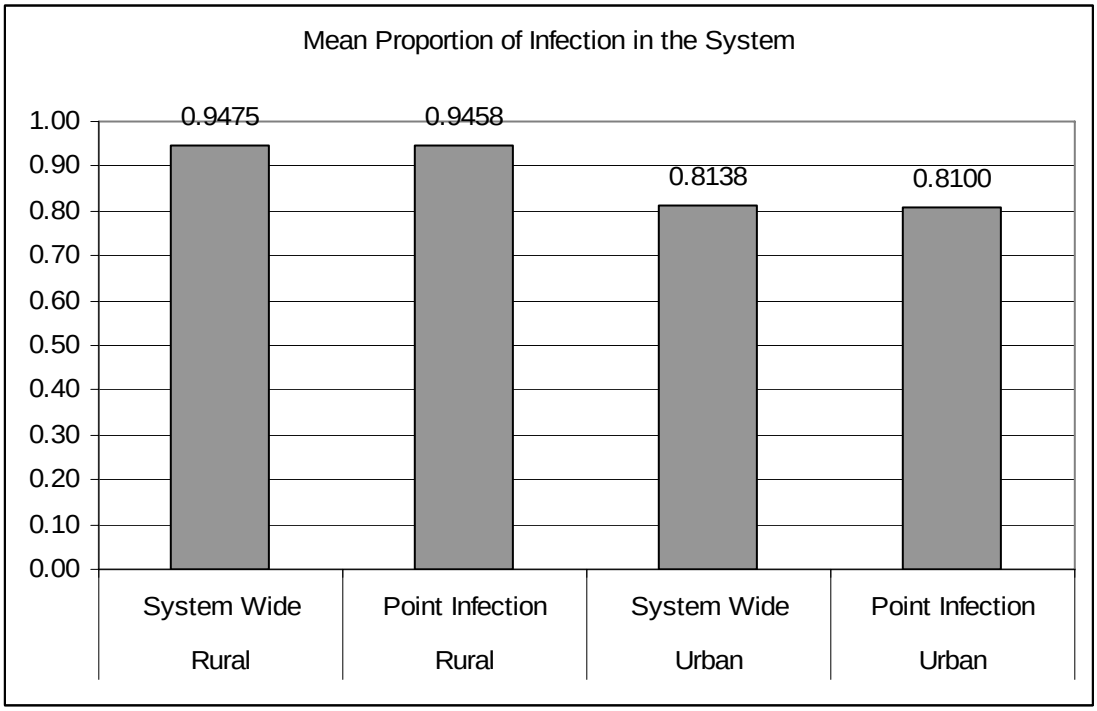


Figure 4.4. Mean proportion of the system infected with *F. tularensis*. The four treatments represent landscape (Rural versus Urban) and introduction (System-wide versus Point) differences.

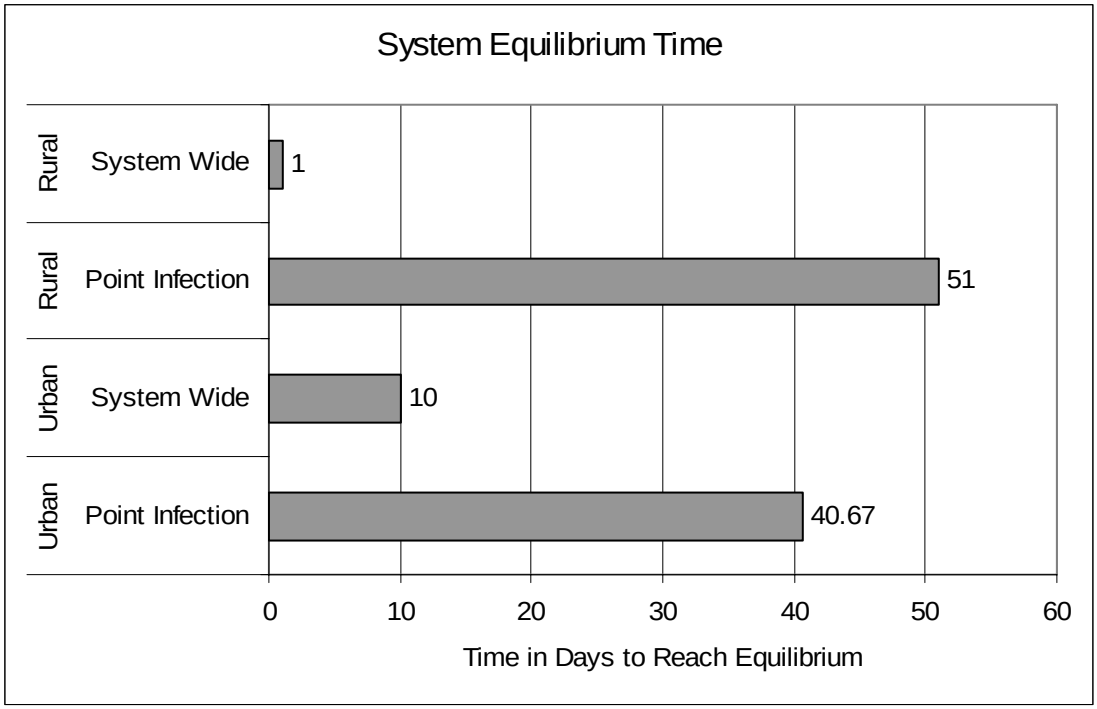


Figure 4.5. Time to equilibrium in the system infected with *F. tularensis*. Horizontal bars represent time in days for the system to reach equilibrium (steady state).

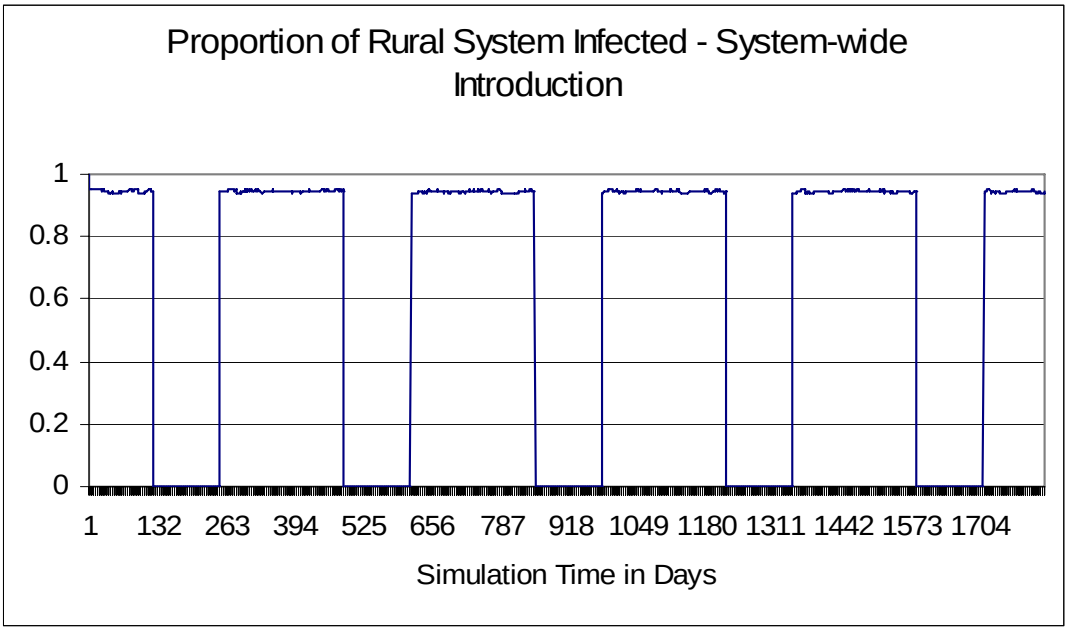


Figure 4.6. Proportion of rural area infected with *F. tularensis* – system-wide introduction: five year simulation, all components included in the simulation.

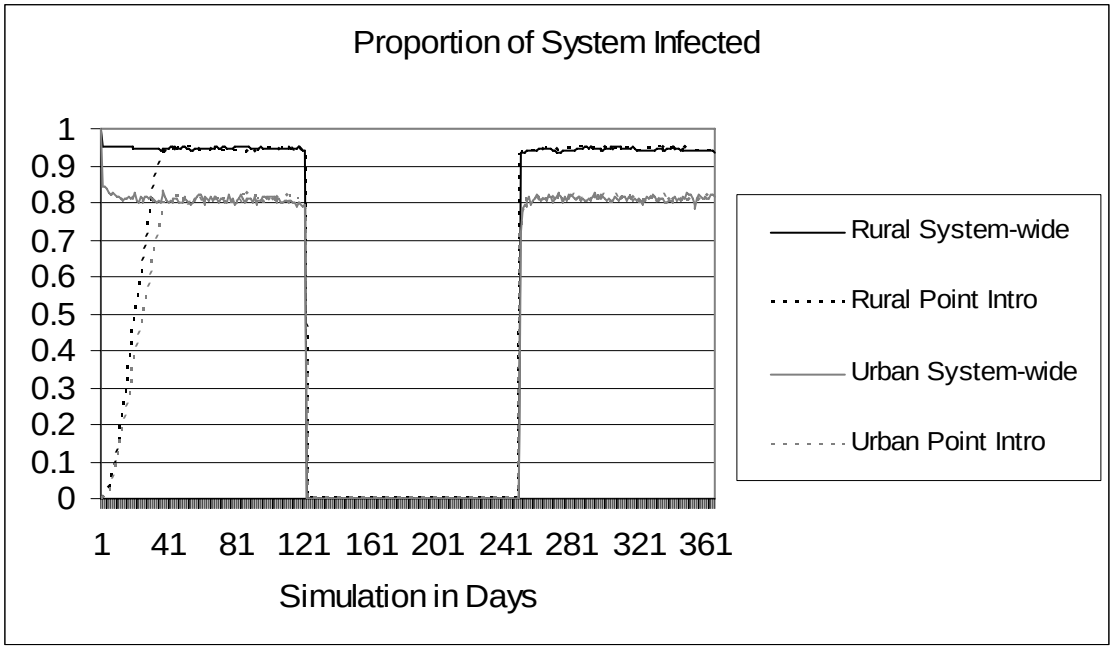
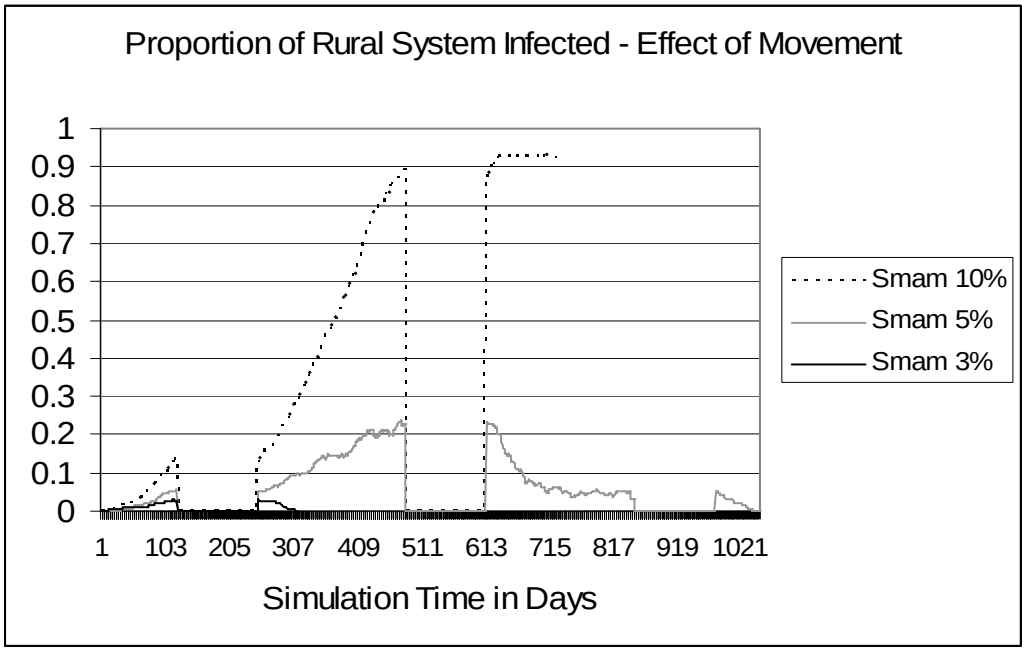
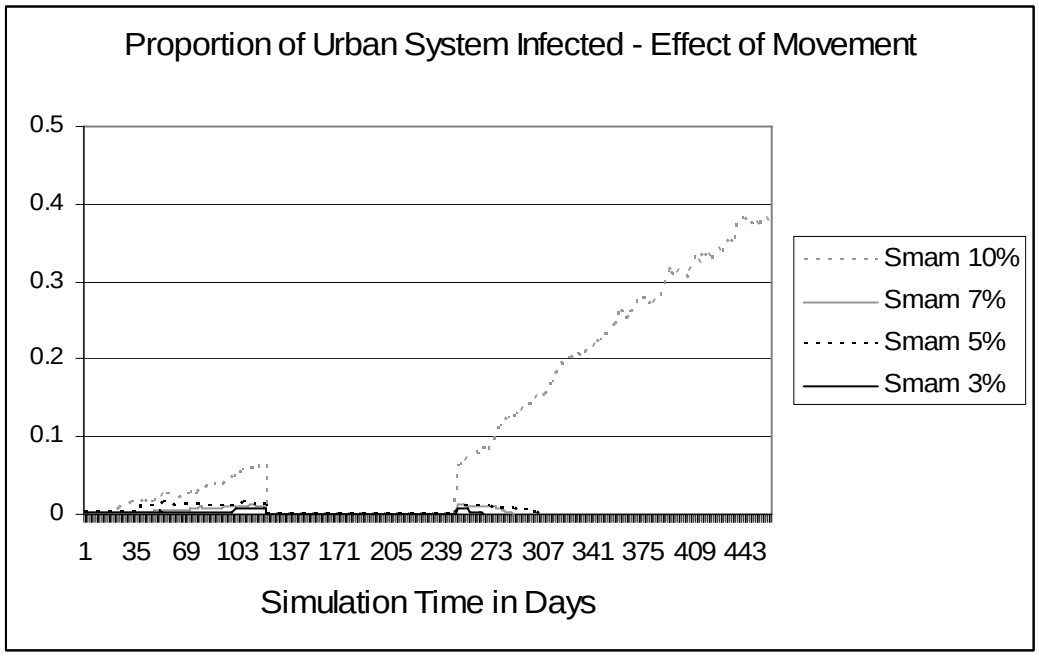


Figure 4.7. Proportion of area infected with *F. tularensis* – four treatments: rural, urban, system-wide, and point. One year simulation, all components included.



A



B

Figure 4.8. Effect of movement on proportion of system infected with *F. tularensis*. Movement of *F. tularensis* is due to Smam only (Deer removed). 4.8A shows effect on rural landscape. 4.8B shows effect on urban landscape, note the change in scale of proportion. *F. tularensis* was introduced in simulations as a point infection.

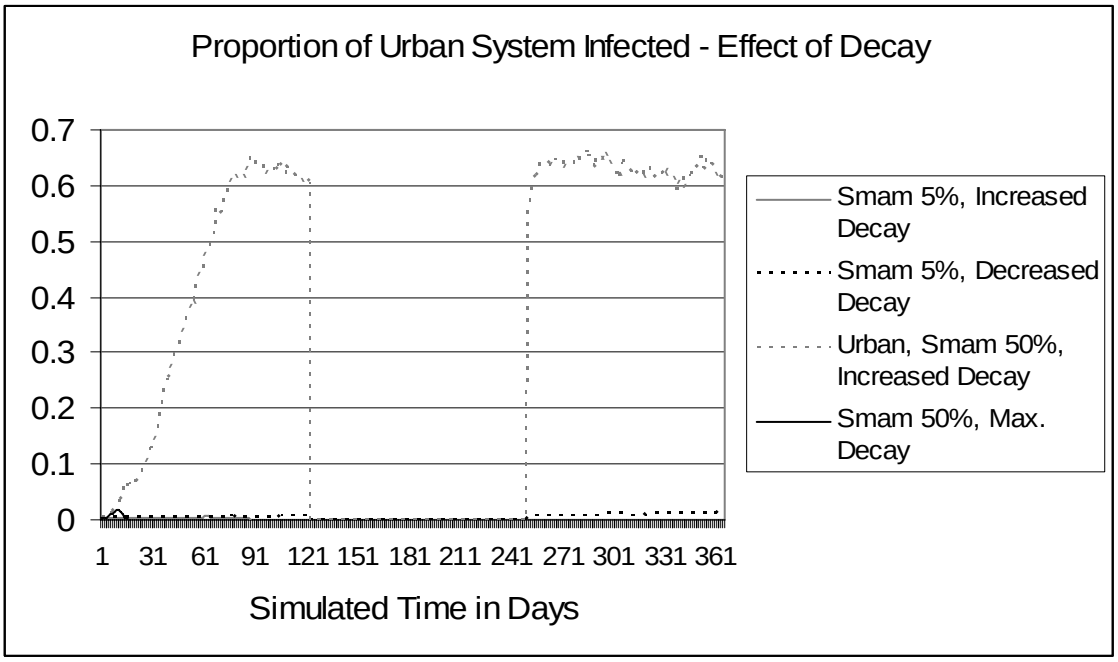


Figure 4.9. Effect of decay on proportion of system infected . Movement was evaluated at two levels (5% and 50% Smam) and decay rates were either increased or decreased.

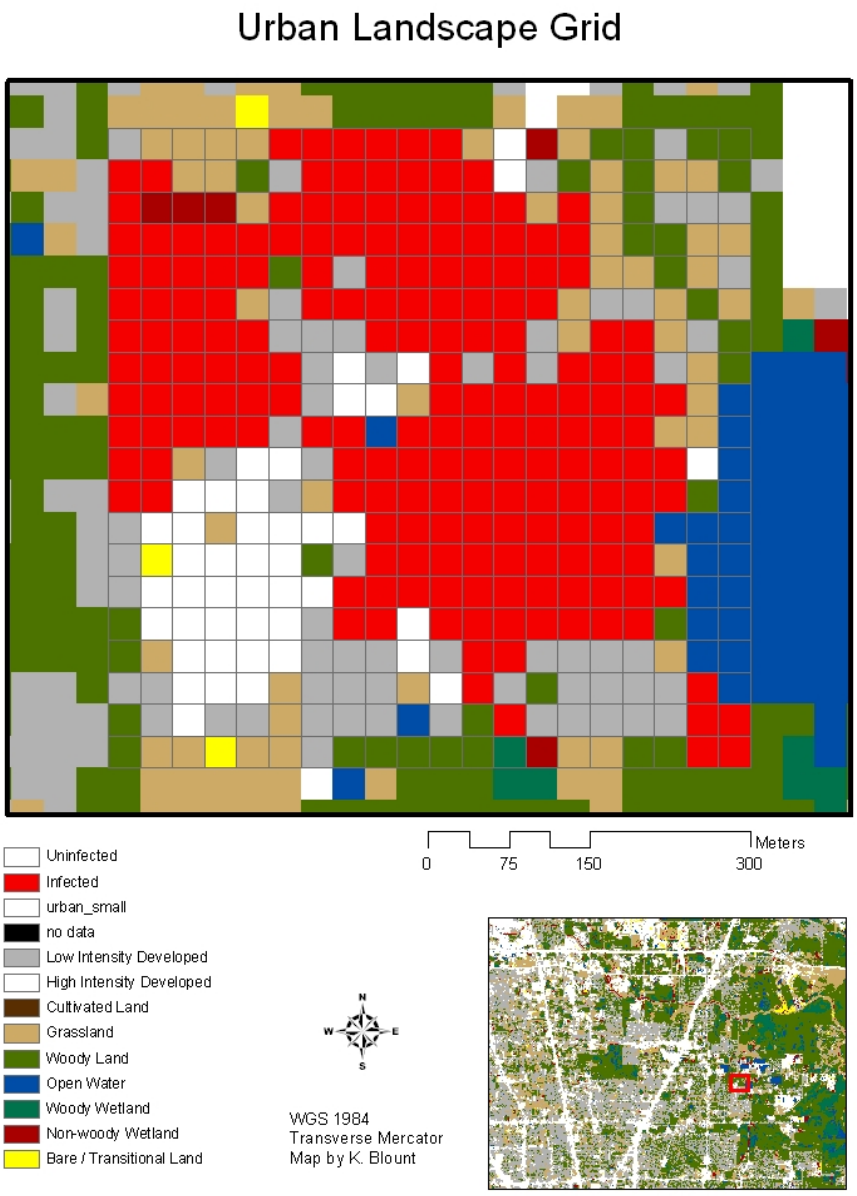


Figure 4.10. Model output for small urban landscape simulating *F. tularensis* infection at the end of a two year simulation. The model parameters were: Point infection, Deer removed, Smam movement probability 0.1.

Discussion

Results of the simulations suggested *F. tularensis* can maintain infections indefinitely in the rural landscape (Fig. 4.6). In urban landscapes, *F. tularensis* can maintain infections in suitable habitats for multiple years, depending on movement of hosts and quality of the habitat (Fig. 4.10). These results support the potential for disease endemicity in locations with all epidemiological components and suitable off-host environment.

Results of the simulations suggested movement of entities had the greatest impact on total tularemia infection within the system. Once *F. tularensis* was introduced, the movement of deer throughout the simulated world continually moved infected ticks into new areas. If the dispersal methods (i.e., small mammals and/or deer) were removed from the system, the mean proportion of the system infected remained essentially unchanged in rural areas, however, the time required to reach this equilibrium was longer.

Since other variables in the model were held constant throughout each simulation (e.g. decay rates for land classes), the outcome of changes in other parameters was somewhat deterministic. Since the function of deer in the simulation was simply as a dispersal mechanism for ticks, the movement of ticks and the pathogen was driven by deer. This difference in movement potential overshadows the impact and influence of movement potential of small mammals.

Unique to this model was the concept that an individual habitat (cell) was the central focus, not individual biological entities. Even though individuals (vectors and

hosts) are the components within a real system that perpetuate a disease, their roles, value, and exact numbers are difficult to estimate. As my objective was to create a model that could be used quickly to evaluate a given landscape to possibly more than one pathogen (or a modified pathogen with which we know very little), creating a model with dozens of inputs defeated the intended purpose. All host-vector-pathogen interactions were handled collectively as a suite of attributes of a habitat rather than as movements of many separate individuals. As a result of this novel approach, an estimate of a complex disease system using a simple two-parameter model was determined. This approach can be used for other host-vector-pathogen interactions.

CHAPTER V

SUMMARY AND CONCLUSIONS

Although clinical cases are rare, *Francisella tularensis* is present in southeast Texas – and not just in rural areas. This research has shown *F. tularensis* is difficult to find in host animals and arthropod vectors, despite improvements in diagnostics. Even rarer is finding *F. tularensis* in an urban environment, but such was found in this study in a common urban animal, the feral cat. *F. tularensis* was also identified in one ixodid tick but it was not the lone star tick, *Amblyomma americanum*, the vector we expected to produce *F. tularensis*, rather it was found in *A. maculatum*, the Gulf Coast tick. Eisen (2007) recently sent out a timely call for renewed research on tick-borne tularemia, focusing on *A. americanum*. I agree wholeheartedly with the author that it is time once again to address this problem, but a broad net needs to be cast if we hope to make true progress on this disease. Our focus for renewed research on tularemia should consider any and all arthropod vectors, vertebrate and invertebrate reservoirs, and the interaction these entities have with their environment. Possibly the greatest lesson learned from this research with tularemia is to expect the unexpected (this observation would be even more true were the pathogen to be modified and weaponized).

This work has shown that *F. tularensis*, and other tick-borne pathogens, can indeed be found in urban and suburban landscapes. Sprawl, human encroachment on wildlife species, and increasing host and vector ranges are just a few reasons we must abandon our old definitions of diseases like tularemia. Although I do not expect

tularemia foci to become established in downtown Houston, the threat of bioterrorism-related introduction of *F. tularensis* into this area makes it imperative what we investigate the potential for this pathogen to spread in this area. In all likelihood, the epicenter of tularemia in the U.S. will remain the Ozark Mountain region, but we need to better understand what environmental factors make this area is so conducive to the perpetuation of this pathogen. Understanding the environment of a pathogen will help explain why it is present in some areas and absent from others. Answers to the basic ecological questions like these will help us evaluate the potential of *F. tularensis* to become established in areas historically viewed as low risk (urban/suburban) and help predict which areas might be more susceptible to introduction and establishment. The pathogen, vectors, and hosts also will adapt in response to their changing environment, and so should our approach to their study.

Tick-borne diseases are on the increase in the United States (Gubler 1998). The potential for tularemia, and other ixodid transmitted diseases, may be greater than we realize. Results of simulation models suggest tularemia may move from rural areas to urban areas, or vice versa. If the epidemiological components (host, agent, and vector) are present in an urban or suburban area, tularemia may circulate for some period of time. Based on these simulations, the ability of the pathogen to move into areas with a sufficient number of susceptible hosts is paramount. Simulation models like the one developed for this project provide important information for public health authorities. These results help provide guidance for surveillance and control in the event of an outbreak or intentional release of tularemia. These results also enable researchers to ask

questions about components of this disease and the complex system interactions involved. This, in turn, may help guide further field work into this fascinating system.

Even though almost a century has past since tularemia was first described and more than thirty years have gone by since Hopla's last field and laboratory studies on ticks and tularemia (1953, 1955, 1974), the ecology of this disease is only slightly better understood today than it was decades ago. The complexities of *F. tularensis* transmission and maintenance are yet to be solved. Fewer field studies attempt to understand how and why this disease persists in an age of modern medicine and other advances in science. As treatments of diseases improve, the need to understand the underlying mechanisms appears less important. Why search for a cause when there is a cure? This rationale seems reasonable but fails us in the end.

Medicine bears only part of the blame. The advances in chemistry that brought us antibiotics also brought us pesticides. Both have saved countless lives and prevented suffering. Both have been depended on too much and overused with the same result: reduced efficacy due to resistance. Post WWII entomology abandoned the ecosystem approach to pest management with the advent of mass-produced insecticides. Proven non-chemical control practices and basic research were left behind as new formularies and delivery mechanisms were developed for pesticides. The same can be said of treatment for many human diseases. Antibiotics are the "pesticides" of the medical community. Fewer clinicians search for root causes or underlying health problems. Instant gratification may catch these fields of science wanting for answers.

How would the scientific community answer the public if *F. tularensis* or a similar pathogen developed resistance to current antibiotic therapies and emerged again as a common vector-borne disease? Is there a good answer why the most basic information regarding host and vector components are unknown? Many of the same questions posed by McCoy, Francis, Parker, Jellison, Hopla and others are still unanswered today. Rather than view this negatively, I see this as a challenge to a new generation of scientists (entomologists, mammalogists, epidemiologists, microbiologists, veterinarians, physicians) to advance our understanding of tularemia ecology, as well as that of other complex vector-borne diseases.

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